PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 January 2001 (23.01.01)	
International application No. PCT/US00/09587	**Applicant's or agent's file reference** PCT 20430
International filing date (day/month/year) 10 April 2000 (10.04.00)	**Priority date** (day/month/year) 14 April 1999 (14.04.99)
Applicant PETRUKHIN, Konstantin et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 November 2000 (10.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Kiwa Mpay
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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(72) Inventor; and

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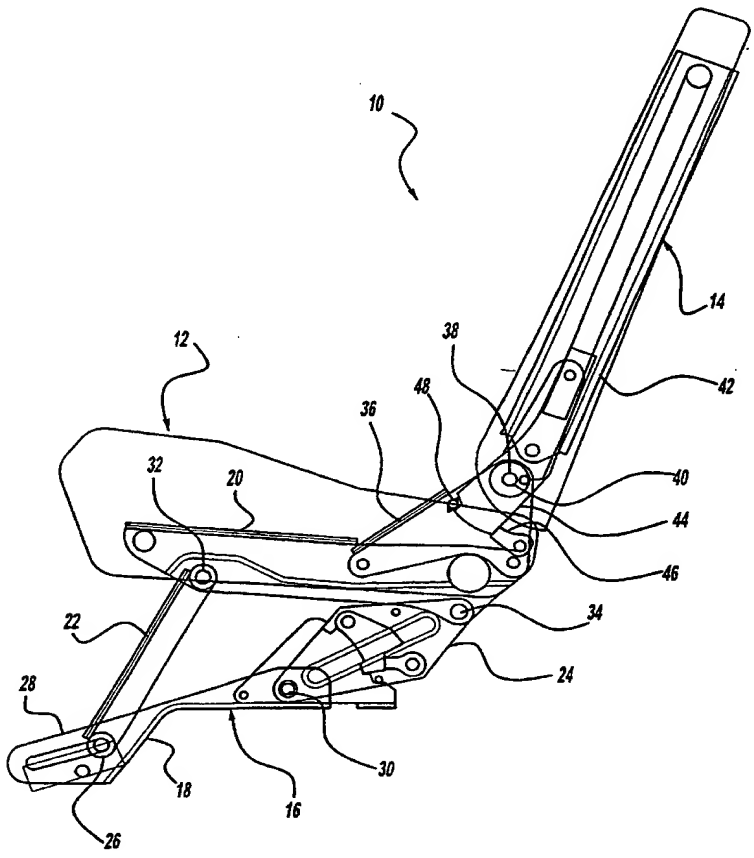
[Continued on next page]

(54) Title: FOLDING VEHICLE SEAT



(57) Abstract: A seat assembly (10) for a vehicle with a seat cushion (12), a seat back (14) connected to the seat cushion, a front link (22) having an upper end and a lower end, and a rear link (24) having an upper end and a lower end. The upper end of the front link is connected to the seat cushion for pivotal movement about a first axis (32), while the lower end of the front link is connected to the vehicle for pivotal movement about a second axis (50). The front link is further connected to either the seat cushion or the vehicle for sliding movement from a first position to a second position. The upper end of the rear link is connected to the seat cushion for pivotal movement about a third axis (34), while the lower end of the rear link is connected to the vehicle for pivotal movement about a fourth axis (30). The seat assembly may be transitioned from a first use position to a second use position by pivoting the seat cushion about the second axis and the fourth axis. The seat assembly may also be transitioned from the first use position to a stowed position by sliding the front link from the first position to the second position and by rotating the seat cushion about the third axis.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT 20430	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/09587	International filing date *(day/month/year)* 10 APRIL 2000	Priority date *(day/month/year)* 14 APRIL 1999
International Patent Classification (IPC) or national classification and IPC IPC(7): C07K 1/00; G01N 33/53; C07K 16/00 and US Cl.: 530/350, 387.1; 435/7.1		
Applicant MERCK & CO., INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 10 NOVEMBER 2000	Date of completion of this report 30 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PATRICK NOLAN Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/09587

I. Basis f the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-40 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 41-45 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-51 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages 1-55 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[X] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readable form.

[X] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/09587

V. Reasoned statem nt under Article 35(2) with regard t novelty, inventiv step or industrial applicability; itati ns and explanati ns supp rting such statement

1. statement

Novelty (N)	**Claims**	1-21	**YES**
	Claims	NONE	**NO**
Inventive Step (IS)	**Claims**	1-21	**YES**
	Claims	NONE	**NO**
Industrial Applicability (IA)	**Claims**	1-21	**YES**
	Claims	NONE	**NO**

2. citations and explanations (Rule 70.7)

Claims 1-21 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the KCNQ5 protein or uses thereof.

NEW CITATIONS

NONE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09587

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 1/00, 16/00; G01N 33/53
US CL : 530/350, 387.1; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 387.1; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST DIALOG EMBASE MEDLINE BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KANANURA et al. The new voltage gated potassium channel KCNQ5 and neonatal convulsions. NeuroReport. June 2000 Vol. 11, No. 9, pages 2063-2067, see entire document.	1-10
Y	WANG et al. KCNQ2 and KCNQ3 Potassium Channel Subunits: Molecular Correlates of the M-Channel. Science. 04 December 1998, Vol. 282, pages 1890-1893, see entire document	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
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Date of the actual completion of the international search 2 JULY 2000	Date of mailing of the international search report 04 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PATRICK NOLAN Telephone No. (703) 308-1235

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INTERNATIONAL APPLICATION PAPERS IN THE APPLICATION FILE :

- ☑ International Application *(RECORD COPY)*
- ☐ Article 19 Amendments
- ☑ PCT/IB/331
- ☑ PCT/IPEA/409 IPER (PCT/IPEA/416 on front)
- ☐ Annexes to 409
- ☐ Priority Document (s) No. _____
- ☐ International Appl. on Double Sided Paper *(COPIES MADE)*
- ☐ Request form PCT/RO/101
- ☑ PCT/ISA/210 - Search Report
- ☐ Search Report References
- ☐ Other : _____

RECEIPTS FROM THE APPLICANT *(other than checked above) :*

- ☑ Basic National Fee (*or authorization to charge*)
- ☑ Description
- ☑ Claims
- ☐ Words in the Drawing Figure(s)
- ☐ Article 19 Amendments
  - ☐ english transl. of annexes NOT present
  - ☐ entered ☐ not entered :
    - ☐ not a page for page substitution
    - ☐ other : _____
- ☐ Annexes to 409
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- ☐ Preliminary Amendment(s) Filed on : 1. _____ 2. _____ 3. _____
- ☐ Information Disclosure Statement(s) Filed on : 1. _____ 2. _____ 3. _____
- ☐ Assignment Document
- ☑ Power of Attorney/ Change of Address
- ☐ Substitute Specification Filed on : 1. _____ 2. _____
- ☐ Verified Small Status Statement (executed)
- ☑ Oath/ Declaration (~~executed~~ Unsigned)
  - ☐ surcharge was paid at the time of filing
- ☐ DNA Diskette
- ☐ Other : 1. _____ 2. _____

NOTES : ☐ I.A. used as Specification ☐ Other :

35 U.S.C. 371 - Receipt of Request (PTO-1390)
Date Acceptable Oath/ Declaration Received
Date of Completion of requirements under 35 U.S.C. 371
102(e) Date
Date of Completion of DO/ EO 906 - Notification of Missing 102(e) Requirements
Date of Completion of DO/ EO 907 - Notification of Acceptance for 102(e) Date
Date of Completion of DO/ EO 911 - Application Accepted Under 35 U.S.C. 111
Date of Completion of DO/ EO 905 - Notification of Missing Requirements
Date of Completion of DO/ EO 916 - Notification of Defective Response
Date of Completion of DO/ EO 903 - Notification of Acceptance
Date of Completion of DO/ EO 909 - Notification of Abandonment

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PETRUKHIN, Konstantin** [RU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CASKEY, C., Thomas** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **LI, Wen** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **METZKER, Michael, L.** [US/US]; 126 Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: CA, JP, US.

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(54) Title: NOVEL HUMAN VOLTAGE-GATED POTASSIUM CHANNEL

(57) Abstract: The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel, KCNQ5, located in a chromosomal region that contains a gene associated with Stargardt-like macular dystrophy, cone-rod macular dystrophy, and Salla disease.

TITLE OF THE INVENTION

NOVEL HUMAN VOLTAGE-GATED POTASSIUM CHANNEL

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel.

BACKGROUND OF THE INVENTION

Voltage-gated potassium channels form transmembrane pores that open in response to changes in cell membrane potential and selectively allow potassium ions to pass through the membrane. Many voltage-gated potassium channels have been identified. They are distinguishable by tissue-specific patterns of expression as well as by electrophysiological and pharmacological properties.

Voltage-gated potassium channels have been shown to be involved in maintaining cell membrane potentials and controlling the repolarization of action potentials in many cells, *e.g.*, neurons, muscle cells, and pancreatic β cells. They are important targets for drug discovery in connection with a variety of diseases.

Functional voltage-gated potassium channels are believed to be tetramers of four alpha subunits, each of which contains six transmembrane spanning segments. The alpha subunits making up a tetramer may be the same (in the case of homotetramers) or may be different (in the case of heterotetramers). The membrane-spanning alpha subunits making up the tetramers may sometimes be associated with additional, beta subunits, which may alter the behavior of the tetramers.

For reviews of voltage-gated potassium channels see Robertson, 1997, Trends Pharmacol. Sci. 18:474-483; Jan & Jan, 1997, J. Physiol. 505:267-282; Catterall, 1995, Ann. Rev. Biochem. 64:493-531.

Macular dystrophy is a term applied to a heterogeneous group of diseases that collectively are the cause of severe visual loss in a large number of people. A common characteristic of macular dystrophy is a progressive loss of central vision resulting from the degeneration of the pigmented epithelium underlying the retinal macula. In many forms of macular dystrophy, the end stage of the disease results in legal blindness. More than 20 types of macular dystrophy are known: e.g., age-related macular dystrophy, Stargardt's and Stargardt-like macular dystrophy, cone-rod dystrophies, atypical vitelliform macular dystrophy (VMD1), Usher Syndrome Type 1B, autosomal dominant neovascular inflammatory vitreoretinopathy, familial exudative vitreoretinopathy, and Best's macular dystrophy. For a review of the macular dystrophies, see Sullivan & Daiger, 1996, Mol. Med. Today 2:380-386.

Cone-rod dystrophies involve an initial loss of cone photoreceptors followed by the degeneration of rod photoreceptors. This loss of photoreceptors can lead to blindness. Cone-rod dystrophies appear to be a heterogeneous group of inherited disorders for which multiple chromosomal locations have been implicated (Evans et al., 1994, Nature Genet. 6:210-213; Kelsell et al., 1997, Hum. Mol. Genet. 6:597-600). In particular, Kelsell et al., 1998, Am. J. Hum. Genet. 63:274-279 found a candidate gene (CORD7) located at chromosome 6q in a four-generation British family affected with cone-rod dystrophy. A marker in 6q, D6S280, showed a high LOD score of 3.31 (at genetic distance = 0).

Stargardt-like macular dystrophy is an inherited, dominant retinal disease. Affected individuals have normal vision in early childhood but show impaired central vision either in late childhood or early adulthood. The first observable characteristics of the disease are flecks seen in the macula. This is followed by central atrophy, resulting in visual acuity decreasing to 20/200 or worse (Stone et al., Arch. Ophthalmol. 112:765-772 [Stone]). Stone mapped a gene responsible for Stargardt-like macular dystrophy to chromosome 6q. The marker D6S280 was observed by Stone to have the high LOD score of 5.5 (at genetic distance = 0).

Cone-rod dystrophy and Stargardt-like macular dystrophy appear different from a clinical perspective. For example, Stargardt-like macular dystrophy

generally begins in childhood and involves white/yellow flecks in the retina while cone-rod dystrophy is an adult-onset disorder in which no flecks are present. Despite such clinical differences, both diseases may be caused by mutations in the same gene. It is not uncommon for different mutations in a single gene to give rise to clinically different disorders. For example, depending upon the particular mutation, mutations in the peripherin/RDS gene can give rise to either butterfly-shaped pigment dystrophy of the fovea, retinitis pigmentosa, pattern dystrophy, flavus maculatus, macular dystrophy, or central areolar choroidal dystrophy (Nichols et al., 1993, Nature Genet. 3:202-207; Weleber et al., 1993, Arch. Ophtalmol. 111:1531-1542; Wells et al., 1993, Nature Genet. 3:213-218; Reig et al., 1995, Ophthalmic. Genet. 16:39-44).

While studies of macular dystrophies such as cone-rod dystrophy or Stargardt-like macular dystrophy are valuable in themselves, such studies are also valuable in that they are expected to shed light on age-related macular degeneration (AMD). AMD is the leading cause of severe visual loss in older individuals. Genetic factors apparently play a role in AMD (Hyman et al., 1983, Am. J. Epidemiol. 118:213-227; Gass, 1973, Arch. Ophthamol. 90:206-217). It is believed likely that mild allelic variations of such earlier-onset diseases as cone-rod dystrophy and Stargardt-like macular dystrophy are responsible for some cases of AMD. Thus, understanding and developing treatments for these earlier-onset diseases should prove valuable with respect to AMD as well.

Salla disease is a recessive condition characterized by early-onset psychomotor retardation and ataxia that involves defects in the lysosomal transport of sialic acid. Leppänen et al., 1996, Genomics 37:62-67 (Leppänen) located the gene for Salla disease in the immediate vicinity of the marker D6S280. Leppänen screened a PAC library with the marker D6S280 and obtained three positive clones, among which were PAC 141B1 and PAC 224H23, strongly suggesting that the gene for Salla disease is present on these PACs.

SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel, KCNQ5, located in a chromosomal region that contains a gene associated with Stargardt-like macular dystrophy, cone-rod macular dystrophy, and Salla disease.

The present invention includes genomic KCNQ5 DNA as well as cDNA that encodes the KCNQ5 protein. The human genomic KCNQ5 DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The human cDNA encoding KCNQ5 protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is KCNQ5 protein encoded by the novel DNA sequences. The human KCNQ5 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3. Methods of expressing KCNQ5 protein in recombinant systems are provided as well as methods of identifying activators and inhibitors of KCNQ5 protein function. Also provided are diagnostic methods that detect carriers of mutant KCNQ5 genes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-AO shows the genomic DNA sequence of human KCNQ5 (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon in exon 1 and the stop TAA codon in exon 14 are shown in bold italics. The D6D280 genetic marker and a phosphoglycerate pseudogene are underlined in bold. The exact lengths of the gaps between exons 1 and 2, 2 and 3, 10 and 11, 11 and 12, 12 and 13, and 13 and 14 are unknown. These gaps are represented as runs of ten bold ns for the sake of convenience.

Figure 2A-E shows the nucleotide sequence (SEQ.ID.NO.:2) and encoded amino acid sequence (SEQ.ID.NO.:3) of human KCNQ5 cDNA. The ATG start codon is at position 138; the TAA stop codon is at position 2,676.

Figure 3A shows the results of a Northern blot of KCNQ5 mRNA expression in various human tissues. Figure 3B shows the results of RT-PCR analysis of KCNQ5 mRNA expression in various human tissues.

Figure 4A shows a sequence alignment of human KCNQ5 protein (SEQ.ID.NO.:3) with human KCNQ4 protein (SEQ.ID.NO.:4). The consensus sequence shown is (SEQ.ID.NO.:5). Figure 4B-C shows a multiple sequence alignment between human KCNQ5 protein (SEQ.ID.NO.:3), human KCNQ1 protein (SEQ.ID.NO.:43), human KCNQ2 protein (SEQ.ID.NO.:6), human KCNQ3 protein (SEQ.ID.NO.:7), and human KCNQ4 protein (SEQ.ID.NO.:4). The consensus sequence shown is (SEQ.ID.NO.:8).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a KCNQ5 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- KCNQ5 proteins. Whether a given KCNQ5 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a KCNQ5 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- KCNQ5 nucleic acids. Whether a given KCNQ5 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

A polypeptide has "substantially the same biological activity as KCNQ5" if that polypeptide conducts a voltage-gated potassium current when expressed in appropriate cell types and has an amino acid sequence that is at least about 50% identical to SEQ.ID.NO.:3 when measured by such standard programs as BLAST or FASTA.

The present invention relates to the identification and cloning of KCNQ5, a gene encoding a novel voltage-gated potassium channel. The human KCNQ5 gene is located on chromosome 6q14, in a chromosomal region that contains genes that have been linked with the occurrence of at least three diseases: Stargardt-like macular dystrophy, cone-rod dystrophy, and Salla disease.

The human KCNQ5 gene is present on PAC clones from chromosomal region 6q14. PAC141B1 was sequenced and KCNQ5 was found based on homology between the genomic sequences of KCNQ5 present in PAC 141B1 and the sequences of known potassium channel genes. PAC 141B1 is available from Research Genetics, Inc., Huntsville, AL, as an individual clone from the RPCI4,5,6 Library (catalog number CTLI.C). Using PCR primers derived from the KCNQ5 sequence, a cDNA sequence representing the coding region as well as a large portion of the 3'-UTR of KCNQ5 was isolated from a human fetal brain cDNA library. Comparison of this cDNA clone with the genomic sequences present in PAC141B1, as well as KCNQ5 sequences found in PAC224H23, showed that exons 3-11 and portions of flanking intronic regions are present in PAC141B1. Exon 2 and flanking intronic regions were found in PAC224H23, while the rest of the KCNQ5 gene (exons 1, 12-14, and flanking intronic regions) was recovered from total human genomic DNA by using cDNA primers and a GenomeWalker kit from Clontech, Palo Alto, CA.

PAC141B1 and PAC224H23 are located in the region of the Salla disease gene (Leppänen et al., 1996, Genomics 37:62-67). PAC141B1 contains the polymorphic genetic marker D6S280 that is located in intron 3 of the KCNQ5 gene between exons 3 and 4 (Figure 1). D6S280 is the marker that detects the maximum LOD score of 5.5 (at genetic distance = 0) in families with Stargardt-like macular dystrophy (Stone et al., Arch. Ophthalmol. 112:765-772). D6S280 also detects a LOD score of 3.31 (at genetic distance = 0) in families with cone-rod dystrophy (Kelsell et al., 1998, Am. J. Hum. Genet. 63:274-279). These LOD scores indicate that D6S280 is very closely linked to, and probably is within, the gene for Stargardt-like macular dystrophy and cone-rod dystrophy. In view of these findings, it is likely that KCNQ5 is involved in Salla disease, Stargardt-like macular dystrophy, and cone-rod dystrophy.

That KCNQ5 should be involved with these three diseases is consistent with its expression pattern (see Figure 3A-B) which shows that KCNQ5 is expressed predominately in the retina and brain, in addition to being expressed in the

skeletal muscle. Stargardt-like macular dystrophy and cone-rod dystrophy are inherited retinal diseases while Salla disease is a disorder that is characterized by early onset psychomotor retardation and ataxia.

Bioinformatic analysis revealed a striking homology of the KCNQ5 protein to a group of voltage gated potassium channels (KCNQ1, KCNQ2, KCNQ3, and KCNQ4; see Figure 4A-B). All of the typical amino acid motifs of these potassium channels are preserved in KCNQ5. A Kyte-Doolittle algorithm analysis predicts a transmembrane organization for KCNQ5 that is typical for this group of potassium channels. Mutations in members of this family of potassium channels have been shown to result in inherited disease (KCNQ2 and KCNQ3, epilepsy [Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690]; KCNQ4, a form of nonsyndromic dominant deafness [Kubisch et al., 1999, Cell 96:437-446], KCNQ1, congenital long QT syndrome which causes cardiac arrhythmias and sudden death [Splawski et al., 1997, N. Engl. J. Med. 336:1562-1567]).

The present invention provides DNA encoding KCNQ5 that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding KCNQ5. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 14 exons. These exons collectively have an open reading frame that encodes a protein of 846 amino acids.

The present invention includes cDNA encoding KCNQ5 protein. Such a cDNA is shown in Figure 2 as SEQ.ID.NO.:2. The present invention therefore includes DNA comprising the nucleotide sequence SEQ.ID.NO.:2. The DNA can be isolated or substantially free of other DNA sequences.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 138-2,675 of SEQ.ID.NO.:2. Also included are recombinant DNA molecules having a nucleotide sequence comprising positions 138-2,675 of SEQ.ID.NO.:2 and isolated DNA molecules having a nucleotide sequence comprising positions 138-2,675 of SEQ.ID.NO.:2.

The novel DNA sequences of the present invention encoding KCNQ5, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which KCNQ5 is not naturally linked, to form "recombinant DNA molecules" encoding KCNQ5. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NO:1 or SEQ.ID.NO:2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the KCNQ5 protein where the nucleotide

sequence of the synthetic DNA differs significantly from the nucleotide sequences of SEQ.ID.NO:2, but still encodes the same KCNQ5 protein as SEQ.ID.NO:2. Such synthetic DNAs are intended to be within the scope of the present invention.

Mutated forms of SEQ.ID.NO:1 or SEQ.ID.NO:2 are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NO:1 or SEQ.ID.NO:2 which give rise to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration are within the scope of the present invention.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding KCNQ5 protein. Such recombinant host cells can be cultured under suitable conditions to produce KCNQ5 protein. An expression vector containing DNA encoding KCNQ5 protein can be used for expression of KCNQ5 protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, amphibian cells such as *Xenopus* oocytes, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cells and cell lines which are suitable for recombinant expression of KCNQ5 protein and which are widely mavailable, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), ARPE-19 human retinal pigment epithelium (ATCC CRL-2302), *Xenopus* melanophores, and *Xenopus* oocytes.

A variety of mammalian expression vectors can be used to express recombinant KCNQ5 in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Another suitable vector is the PT7TS oocyte expression vector. Following expression in recombinant cells, KCNQ5 can

be purified by conventional techniques to a level that is substantially free from other proteins.

Certain voltage-gated potassium channel subunits have been found to require the expression of other voltage-gated potassium channel subunits as "chaperones" in order to be properly expressed at high levels and inserted in membranes. For example, co-expression of KCNQ3 appears to enhance the expression of KCNQ2 in *Xenopus* oocytes (Wang et al., 1998, Science 282:1890-1893). Also, some voltage-gated potassium channel Kv1α subunits require other related alpha subunits or Kvβ2 subunits (Shi et al., 1995, Neuron 16:843-852). Accordingly, the recombinant expression of the KCNQ5 protein may under certain circumstances benefit from the co-expression of other voltage-gated potassium channel proteins and such co-expression is intended to be within the scope of the present invention.

The present invention includes KCNQ5 protein substantially free from other proteins. The amino acid sequence of the full-length KCNQ5 protein is shown in Figure 2 as SEQ.ID.NO.:3. Thus, the present invention includes KCNQ5 protein substantially free from other proteins having the amino acid sequence SEQ.ID.NO.:3. The present invention also includes isolated KCNQ5 protein having the amino acid sequence SEQ.ID.NO.:3.

Mutated forms of KCNQ5 proteins are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NO:3 that give rise to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration are within the scope of the present invention.

As with many proteins, it is possible to modify many of the amino acids of KCNQ5 and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified KCNQ5 proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as KCNQ5. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO:3 wherein the polypeptides still retain substantially the same biological activity as

KCNQ5. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO:3 wherein the polypeptides still retain substantially the same biological activity as KCNQ5. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in KCNQ5 is also present in the corresponding position of any one of KCNQ1, KCNQ2, KCNQ3, or KCNQ4 (see Figure 4A-B).

The KCNQ5 proteins of the present invention may contain post-translational modifications, *e.g.*, covalently linked carbohydrate, phosphorylation, myristoylation, *etc.*.

The present invention also includes chimeric KCNQ5 proteins. Chimeric KCNQ5 proteins consist of a contiguous polypeptide sequence of at least a portion of KCNQ5 protein fused to a polypeptide sequence of a non-KCNQ5 protein.

The present invention also includes isolated forms of KCNQ5 proteins and KCNQ5 DNA. Use of the term "isolated" indicates that KCNQ5 protein or KCNQ5 DNA has been removed from its normal cellular environment. Thus, an isolated KCNQ5 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated KCNQ5 protein is the only protein present. but instead means that an isolated KCNQ5 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the KCNQ5 protein. Thus, a KCNQ5 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated KCNQ5 protein."

It is known that other members of the family of potassium channels to which KCNQ5 belongs can interact to form heteromeric structures resulting in functional potassium channels. For example, KCNQ2 and KCNQ3 can assemble to form a heteromeric functional potassium channel (Wang et al., 1998, Science 282:1890-1893). Accordingly, it is believed likely that KCNQ5 will also be able to form heteromeric structures with other proteins where such heteromeric structures constitute functional potassium channels. Thus, the present invention includes such heteromers comprising KCNQ5. Preferred heteromers are those in which KCNQ5 forms heteromers with at least one of KCNQ1, KCNQ2, KCNQ3, or KCNQ4.

A cDNA fragment encoding full-length KCNQ5 can be isolated from a human retinal or brain cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for KCNQ5 shown in Figure 2 as SEQ.ID.NO.:2. Suitable primer pairs would be, *e.g.*:

5'-GGGGGCCCGGATGAGCC-3' (SEQ.ID.NO.:9) and

5'-GAAGAACTTATTTCAGTTTGA-3' (SEQ.ID.NO.:10)

The above primers are meant to be illustrative only; one skilled in the art would readily be able to design other suitable primers based upon SEQ.ID.NO.:2. Such primers could be produced by methods of oligonucleotide synthesis that are well known in the art.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM $MgCl_2$, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press .

A suitable cDNA library from which a clone encoding KCNQ5 can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA) or human fetal brain 5-stretch plus cDNA library (catalog number HL5024t, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of 846 amino acids (SEQ.ID.NO.:3) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). KCNQ5 protein can then be produced by transferring an expression

vector encoding KCNQ5 or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. KCNQ5 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding KCNQ5 can be isolated from a cDNA library using as a probe oligonucleotides specific for KCNQ5 and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for KCNQ5 and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of KCNQ5 shown in Figure 2 as SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the KCNQ5 gene can be obtained from commercially available human PAC or BAC libraries available from Research Genetics, Huntsville, AL. PAC clones containing the KCNQ5 gene (*e.g.*, PAC141B1, PAC224H23) are commercially available from Research Genetics, Huntsville, AL (catalog number for individual PAC clones is RPCI.C). Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the KCNQ5 can be isolated, using probes based upon the KCNQ5 sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*,1994, Nature Genet. 6:84-89).

The novel DNA sequences of the present invention can be used in various diagnostic methods relating to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. The present invention provides diagnostic methods for determining whether a patient carries a mutation in the KCNQ5 gene that predisposes that patient toward the development of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. In broad terms, such methods comprise determining the DNA sequence of a region of the KCNQ5 gene from the patient and comparing that sequence to the sequence from the corresponding region of the KCNQ5 gene from a non-affected person, *i.e.*, a person who does not suffer from Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration, where a difference in sequence between the DNA sequence of the KCNQ5 gene from the

patient and the DNA sequence of the KCNQ5 gene from the non-affected person indicates that the patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

Such methods of diagnosis may be carried out in a variety of ways. For example, one embodiment comprises:

(a) providing PCR primers from a region of the KCNQ5 gene;

(b) performing PCR on a DNA sample from the patient to produce a PCR fragment from the patient;

(c) performing PCR on a control DNA sample comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO.:2 to produce a control PCR fragment;

(d) determining the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment;

(e) comparing the nucleotide sequence of the PCR fragment from the patient to the nucleotide sequence of the control PCR fragment;

where a difference between the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment indicates that the patient has a mutation in the KCNQ5 gene and thus is likely to have Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

In a particular embodiment, the PCR primers are from a region of the KCNQ5 gene where it is suspected that a patient harbors a mutation. In a particular embodiment, the PCR primers are from the coding region of the KCNQ5 gene, *i.e.*, from the coding region of SEQ.ID.NO:1 or SEQ.ID.NO:2. In a particular embodiment, the PCR primers amplify a region that includes the marker D6S280.

In a particular embodiment, the DNA sample from the patient is cDNA that has been prepared from an RNA sample from the patient. In another embodiment, the DNA sample from the patient is genomic DNA. In a particular embodiment, the control DNA sample is DNA from a person who does not have Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are determined by DNA sequencing.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are compared by direct comparison after DNA sequencing. In another embodiment, step (d) is omitted and the comparison in step (e) is made by a process that includes hybridizing the PCR fragment from the patient and the control PCR fragment and then using an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly. Such an endonuclease is, *e.g.*, S1. In this embodiment, the conversion of the PCR fragment from the patient to smaller fragments after endonuclease treatment indicates that the patient carries a mutation in the KCNQ5 gene. In such embodiments, it may be advantageous to label (radioactively, enzymatically, immunologically, *etc.*) the PCR fragment from the patient or the control PCR fragment.

The present invention provides a method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

(a) obtaining an RNA sample from the patient;

(b) performing reverse transcription-PCR (RT-PCR) on the RNA sample using primers that span a region of the coding sequence of the KCNQ5 gene to produce a PCR fragment from the patient where the PCR fragment from the patient has a defined length, the length being dependent upon the identity of the primers that were used in the RT-PCR;

(c) hybridizing the PCR fragment to DNA comprising a sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO.:2, or to portions of SEQ.ID.NO:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels, to form a hybrid;

(d) treating the hybrid produced in step (c) with an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly;

(e) determining whether the endonuclease cleaved the hybrid by determining the length of the PCR fragment from the patient after endonuclease treatment where a reduction in the length of the PCR fragment from the patient after endonuclease treatment indicates that the patient carries a mutation in the KCNQ5 gene.

In a variation of the above-described method, instead of determining the length of the PCR fragment from the patient after endonuclease treatment, the

length of the DNA comprising a sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO.:2, or the DNA comprising portions of SEQ.ID.NO:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels is determined after endonuclease treatment. In such a variation, a reduction in the length of the DNA comprising a sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO.:2, or the DNA comprising portions of SEQ.ID.NO:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels indicates that the patient carries a mutation in the KCNQ5 gene.

The present invention provides a method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

(a) making cDNA from an RNA sample from the patient;

(b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

(c) performing PCR on the cDNA to produce a PCR fragment from the patient;

(d) determining the nucleotide sequence of the PCR fragment from the patient;

(e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

The present invention provides a method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

(a) preparing genomic DNA from the patient;

(b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

(c) performing PCR on the genomic DNA to produce a PCR fragment from the patient;

(d) determining the nucleotide sequence of the PCR fragment from the patient;

(e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

The present invention also provides oligonucleotide probes, based upon the sequences of SEQ.ID.NO:1 or SEQ.ID.NO:2, that can be used in diagnostic methods related to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. In particular, the present invention includes DNA oligonucleotides comprising at least about 10, 15, or 18 contiguous nucleotides of a sequence selected from the group consisting of: SEQ.ID.NO:1 and SEQ.ID.:NO.2 where the oligonucleotide probe comprises no stretch of contiguous nucleotides longer than 5 of a sequence selected from the group consisting of: SEQ.ID.NO:1 and SEQ.ID.:NO.2 other than the said at least about 10, 15, or 18 contiguous nucleotides. The oligonucleotides can be substantially free from other nucleic acids. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the diagnostic utilities described above, the present invention makes possible the recombinant expression of the KCNQ5 protein in various cell types. Such recombinant expression makes possible the study of this protein so that its biochemical activity and its role in Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration can be elucidated.

The present invention also makes possible the development of assays which measure the biological activity of the KCNQ5 protein. Such assays using recombinantly expressed KCNQ5 protein are especially of interest. Assays for KCNQ5 protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of KCNQ5 protein. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to treat patients having Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. In versions of the above-described assays, mutant KCNQ5 proteins are used and inhibitors or activators of the activity of the mutant KCNQ5 proteins are identified.

Preferred cell lines for recombinant expression of KCNQ5 are those which do not express endogenous potassium channels (*e.g.*, CV-1, NIH-3T3). Such

cell lines can be loaded with ^{86}Rb, an ion which can pass through potassium channels. The ^{86}Rb-loaded cells can be exposed to collections of substances (*e.g.*, combinatorial libraries, natural products) and those substances that are able to alter ^{86}Rb efflux identified. Such substances are likely to be activators or inhibitors of KCNQ5.

The present invention includes a method of identifying activators or inhibitors of KCNQ5 comprising:

(a) recombinantly expressing KCNQ5 protein or mutant KCNQ5 protein in a host cell;

(b) measuring the biological activity of KCNQ5 protein or mutant KCNQ5 protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein;

where a change in the biological activity of the KCNQ5 protein or the mutant KCNQ5 protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein.

In particular embodiments, the biological activity is the production of a voltage-gated potassium current, or efflux of ^{86}Rb.

In particular embodiments, a vector encoding KCNQ5 is transferred into *Xenopus* oocytes in order to cause the expression of KCNQ5 protein in the oocytes. Alternatively, RNA encoding KCNQ5 protein can be prepared *in vitro* and injected into the oocytes, also resulting in the expression of KCNQ5 protein in the oocytes. Following expression of KCNQ5 in the oocytes, membrane currents are measured after the transmembrane voltage is changed in steps. A change in membrane current is observed when the KCNQ5 channels opens, allowing potassium ion flow. Similar oocytes studies were reported for KCNQ2 and KCNQ3 potassium channels in Wang et al., 1998, Science 282:1890-1893.

Inhibitors of KCNQ5 can be identified by exposing the oocytes expressing KCNQ5 to collections of substances and determining whether the substances can block or diminish the membrane currents observed in the absence of the substance.

Accordingly, the present invention provides a method of identifying inhibitors of KCNQ5 comprising:

(a) expressing KCNQ5 protein in *Xenopus* oocytes;

(b) changing the transmembrane potential of the oocytes in the presence and the absence of a substance suspected of being an inhibitor of KCNQ5;

(c) measuring membrane potassium currents following step (b);

where if the potassium membrane currents measured in step (c) are greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of KCNQ5.

The present invention also includes assays for the identification of activators and inhibitors of KCNQ5 that are based upon FRET between a first and a second fluorescent dye where the first dye is bound to one side of the plasma membrane of a cell expressing KCNQ5 and the second dye is free to shuttle from one face of the membrane to the other face in response to changes in membrane potential. In certain embodiments, the first dye is impenetrable to the plasma membrane of the cells and is bound predominately to the extracellular surface of the plasma membrane. The second dye is trapped within the plasma membrane but is free to diffuse within the membrane. At normal (*i.e.*, negative) resting potentials of the membrane, the second dye is bound predominately to the inner surface of the extracellular face of the plasma membrane, thus placing the second dye in close proximity to the first dye. This close proximity allows for the generation of a large amount of FRET between the two dyes. Following membrane depolarization, the second dye moves from the extracellular face of the membrane to the intracellular face, thus increasing the distance between the dyes. This increased distance results in a decrease in FRET, with a corresponding increase in fluorescent emission derived from the first dye and a corresponding decrease in the fluorescent emission from the second dye. See figure 1 of González & Tsien, 1997, Chemistry & Biology 4:269-277. See also González & Tsien, 1995, Biophys. J. 69:1272-1280 and U.S. Patent No. 5,661,035.

In certain embodiments, the first dye is a fluorescent lectin or a fluorescent phospholipid that acts as the fluorescent donor. Examples of such a first dye are: a coumarin-labeled phosphatidylethanolamine (*e.g.*, N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-ethanolamine) or N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); a fluorescently-labeled lectin (*e.g.*, fluorescein-labeled wheat germ agglutinin). In certain embodiments, the second dye is an oxonol that acts as the fluorescent acceptor. Examples of such a second dye are: bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols (*e.g.*, bis(1,3-dihexyl-2-

thiobarbiturate)trimethineoxonol) or pentamethineoxonol analogues (*e.g.*, bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; or bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol). See González & Tsien, 1997, Chemistry & Biology 4:269-277 for methods of synthesizing various dyes suitable for use in the present invention. In certain embodiments, the assay may comprise a natural carotenoid, *e.g.*, astaxanthin, in order to reduce photodynamic damage due to singlet oxygen.

The above described assays can be utilized to discover activators and inhibitors of KCNQ5. Such assays will generally utilize cells that express KCNQ5, *e.g.*, by transfection with expression vectors encoding KCNQ5. In assays for inhibitors, such cells will generally have a resting membrane potential that is roughly equal to the threshhold for activation of the KCN5Q channel. This is because most untransfected cells will have membrane potentials that are depolarized relative to the threshhold potential of KCNQ5 channels. Therefore, when KCNQ5 is expressed in these cells, the KCNQ5 channels open. This lets K^+ out of the cells, which tends to hyperpolarize the membrane potential. This closes some of the KCNQ5 channels, leading to relative depolarization. In this way, a steady state develops around the threshhold for activation of the KCNQ5 channel. Inhibitors of KCNQ5 will, therefore, disturb this steady state and depolarize the cell. In assays for activators, KCNQ5 will be transfected into a cell line that also expresses a counteracting, depolarizing current. The membrane potential in these cells will therefore be set by contributions of both the KCNQ5 channel and the endogenous depolarizing current, resulting in a more depolarized resting potential. Ideally, the endogenous current will play the major role in the absence of a KCNQ5 activator. Activators of KCNQ5 will open this channel and increase the contribution of KCNQ5 to the membrane potential relative to the other current and the potential will, therefore, hyperpolarize in response to an activator of KCNQ5. Changes in membrane potential (depolarizations and hyperpolarizations) that are caused by activators and inhibitors of KCNQ5 can be monitored by the assays using FRET described above.

Accordingly, the present invention provides a method of identifying activators of KCNQ5 comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an activator of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a)(1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

The present invention also provides a method of identifying inhibitors of KCNQ5 comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an inhibitor of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is less than the amount of FRET exhibited by the control cells, the substance is an inhibitor of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

In a variation of the assay described above, instead of the transfected cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the KCNQ5 channel is open. This can be done, *e.g.*, by variation of the external K+ concentration in a known manner (*e.g.*, increased concentrations of external K+). If such cells, having open KCNQ5 channels, are exposed to inhibitors of KCNQ5, the KCNQ5 channels will close, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET.

Accordingly, the present invention provides a method of identifying inhibitors of KCNQ5 comprising:

(a) providing cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) adjusting the membrane potential of the cells such that the ion channel formed by KCNQ5 is open;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells;

(d) repeating step (b) and step (c) while the cells are exposed to a substance that is suspected of being an inhibitor of KCNQ5;

where if the amount of FRET exhibited by the cells that are exposed to the substance is less than the amount of FRET exhibited by the cells that have not been exposed to the substance, then the substance is an inhibitor of KCNQ5.

In particular embodiments of the above-described methods, the expression vectors are transfected into the test cells.

In particular embodiments of the above-described methods, KCNQ5 has an amino acid sequence of SEQ.ID.NO.:3.

In particular embodiments of the above-described methods, the first fluorescent dye is selected from the group consisting of: a fluorescent lectin; a fluorescent phospholipid; a coumarin-labeled phosphatidylethanolamine; N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-ethanolamine); N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); and fluorescein-labeled wheat germ agglutinin.

In particular embodiments of the above-described methods, the second fluorescent dye is selected from the group consisting of: an oxonol that acts as the fluorescent acceptor; bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol; bis(1,3-dialkyl-2-thiobarbiturate)quatramethineoxonols; bis(1,3-dialkyl-2-thiobarbiturate)pentamethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol); and bis(1,3-dialkyl-2-thiobarbiturate)hexamethineoxonols.

In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* melanophores, or *Xenopus* oocytes.

In particular embodiments of the above-described methods, the control cells do not comprise item (a)(1) but do comprise items (a)(2) and (a)(3).

In assays to identify activators or inhibitors of KCNQ5, it may be advantageous to co-express another potassium channel, *e.g.*, KCNQ1, KCNQ2, KCNQ3, or KCNQ4, together with KCNQ5, or with an accessory subunit, such as the IsK protein or one of its homologues, in order to form a functional heteromeric potassium channel.

While the above-described methods are explicitly directed to testing whether "a" substance is an activator or inhibitor of KCNQ5, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of KCNQ5. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention.

The present invention includes pharmaceutical compositions comprising activators or inhibitors of KCNQ5 protein that have been identified by the herein-described methods. The activators or inhibitors are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the activators or inhibitors.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where KCNQ5 activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion),

intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The present invention includes a method of treating Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, age-related macular degeneration and other forms of macular degeneration, deafness, epilepsy, and different forms of neuropsychiatric, heart, gastrointestinal, and muscle disorders by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a voltage-gated potassium channel containing the KCNQ5 protein.

When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target ion channel, it is necessary to ensure that the compounds identified are as specific as possible for the target ion channel. To do this, it is necessary to screen the compounds against as wide an array as possible of ion channels that are similar to the target ion channel. Thus, in order to find compounds that are potential pharmaceuticals that interact with ion channel A, it

is not enough to ensure that the compounds interact with ion channel A (the "plus target") and produce the desired pharmacological effect through ion channel A. It is also necessary to determine that the compounds do not interact with ion channels B, C, D, *etc* .(the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). KCNQ5 protein, DNA encoding KCNQ5 protein, and recombinant cells that have been engineered to express KCNQ5 protein have utility in that they can be used as "minus targets" in screens designed to identify compounds that specifically interact with other ion channels. For example, Wang et al., 1998, Science 282:1890-1893 have shown that KCNQ2 and KCNQ3 form a heteromeric potassium ion channel know as the "M-channel." The M-channel is an important target for drug discovery since mutations in KCNQ2 and KCNQ3 are responsible for causing epilepsy (Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690). A screening program designed to identify activators or inhibitors of the M-channel would benefit greatly by the use of KCNQ5 as a minus target.

The present invention also includes antibodies to the KCNQ5 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire KCNQ5 protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, KCNQ5 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, KCNQ5 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case

of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce KCNQ5 polypeptides into the cells of target organs, *e.g.*, the pigmented epithelium of the retina or other parts of the retina. Nucleotides encoding KCNQ5 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, lentivirus, and polio virus based vectors. Alternatively, nucleotides encoding KCNQ5 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with KCNQ5 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate KCNQ5 activity.

The present invention includes processes for cloning orthologues of human KCNQ5 from non-human species. In general, such processes include preparing a PCR primer or a hybridization probe based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2 that can be used to amplify a fragment containing the non-human KCNQ5 (in the case of PCR) from a suitable DNA preparation or to select a cDNA or genomic clone containing the non-human KCNQ5 from a suitable library. A preferred embodiment of this process is a process for cloning the KCNQ5 gene from mouse.

By providing DNA encoding mouse KCNQ5, the present invention allows for the generation of an animal model of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. Such animal models can be generated by making transgenic "knockout" or "knockin" mice containing altered KCNQ5 genes. Knockout mice can be generated in which portions of the mouse KCNQ5 gene have been deleted. Knockin mice can be generated in which mutations that have been shown to lead to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration when present in the human KCNQ5 gene are introduced into the mouse gene. Such knockout and

knockin mice will be valuable tools in the study of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration and will provide important model systems in which to test potential pharmaceuticals or treatments for Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

Accordingly, the present invention includes a method of producing a mouse model of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration comprising:

(a) designing PCR primers or an oligonucleotide probe based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2 for use in cloning the mouse KCNQ5 gene;

(b) using the PCR primers or the oligonucleotide probe to clone at least a portion of the mouse KCNQ5 gene, the portion being large enough to use in making a transgenic mouse;

(c) producing a transgenic mouse having at least one copy of the mouse KCNQ5 gene altered from its native state.

Methods of producing knockout and knockin mice are well known in the art. One method involves the use of gene-targeted ES cells in the generation of gene-targeted transgenic knockout mice and is described in, *e.g.*, Thomas et al., 1987, Cell 51:503-512, and is reviewed elsewhere (Frohman et al., 1989, Cell 56:145-147; Capecchi, 1989, Trends in Genet. 5:70-76; Baribault et al., 1989, Mol. Biol. Med. 6:481-492).

Techniques are available to inactivate or alter any genetic region to virtually any mutation desired by using targeted homologous recombination to insert specific changes into chromosomal genes. Generally, use is made of a "targeting vector," *i.e.*, a plasmid containing part of the genetic region it is desired to mutate. By virtue of the homology between this part of the genetic region on the plasmid and the corresponding genetic region on the chromosome, homologous recombination can be used to insert the plasmid into the genetic region, thus disrupting the genetic region. Usually, the targeting vector contains a selectable marker gene as well.

In comparison with homologous extrachromosomal recombination, which occurs at frequencies approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3} (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395; Smithies et al., 1985, Nature 317: 230-234; Thomas et al., 1986, Cell 44:419-428).

Nonhomologous plasmid-chromosome interactions are more frequent, occurring at levels 10^5-fold (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395) to 10^2-fold (Thomas et al., 1986, Cell 44:419-428) greater than comparable homologous insertion.

To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, Nucleic Acids Res. 16:8887-8903; Kim et al., 1991, Gene 103:227-233). Alternatively, a positive genetic selection approach has been developed in which a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, Proc. Natl. Acad. Sci. USA 86:227-231). One of the most powerful approaches developed for selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists (Mansour et al., 1988, Nature 336:348-352; Capecchi, 1989, Science 244:1288-1292; Capecchi, 1989, Trends in Genet. 5:70-76). The PNS method is more efficient for targeting genes which are not expressed at high levels because the marker gene has its own promoter. Nonhomologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its nonhomologous insertion with herpes drugs such as gancyclovir (GANC) or FIAU (1-(2-deoxy 2-fluoro-B-D-arabinofluranosyl)-5-iodouracil). By this counter-selection, the percentage of homologous recombinants in the surviving transformants can be increased.

Other methods of producing transgenic mice involve microinjecting the male pronuclei of fertilized eggs. Such methods are well known in the art.

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Identification of the human KCNQ5 gene and cDNA cloning

Construction of Libraries for Shotgun Sequencing from PAC Clones

Bacterial strains containing the KCNQ5 PACs (P1 Artificial Chromosomes) were received from Research Genetics (Huntsville, AL). Cells were streaked on Luria-Bertani (LB) agar plates supplemented with the appropriate antibiotic. A single colony was used to prepare a 5-ml starter culture and then 1-L overnight culture in LB medium. The cells were pelleted by centrifugation and PAC DNA was purified by equilibrium centrifugation in cesium chloride-ethidium bromide gradient (Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press). Purified PAC DNA was brought to 50 mM Tris pH 8.0, 15 mM $MgCl_2$, and 25% glycerol in a volume of 2 ml and placed in a AERO-MIST nebulizer (CIS-US, Bedford, MA). The nebulizer was attached to a nitrogen gas source and the DNA was randomly sheared at 10 psi for 30 sec. The sheared DNA was ethanol precipitated and resuspended in TE (10 mM Tris, 1 mM EDTA). The ends were made blunt by treatment with Mung Bean Nuclease (Promega, Madison, WI) at 30°C for 30 min, followed by phenol/chloroform extraction, and treatment with T4 DNA polymerase (GIBCO/BRL, Gaithersburg, MD) in multicore buffer (Promega, Madison, WI) in the presence of 40 uM dNTPs at 16°C. To facilitate subcloning of the DNA fragments, BstX I adapters (Invitrogen, Carlsbad, CA) were ligated to the fragments at 14°C overnight with T4 DNA ligase (Promega, Madison, WI). Adapters and DNA fragments less than 500 bp were removed by column chromatography using a cDNA sizing column (GIBCO/BRL, Gaithersburg, MD) according to the instructions provided by the manufacturer. Fractions containing DNA greater than 1 kb were pooled and concentrated by ethanol precipitation. The DNA fragments containing BstX I adapters were ligated into the BstX I sites of pSHOT II which was constructed by subcloning the BstX I sites from pcDNA II (Invitrogen, Carlsbad, CA) into the BssH II sites of pBlueScript (Stratagene, La Jolla, CA). pSHOT II was prepared by digestion with BstX I restriction endonuclease and purified by agarose gel electrophoresis. The gel purified vector DNA was extracted from the agarose by following the Prep-A-Gene (BioRad, Richmond, CA) protocol. To reduce ligation of

the vector to itself, the digested vector was treated with calf intestinal phosphatase (GIBCO/BRL, Gaithersburg, MD. Ligation reactions of the DNA fragments with the cloning vector were transformed into ultra-competent XL-2 Blue cells (Stratagene, La Jolla, CA), and plated on LB agar plates supplemented with 100 μg/ml ampicillin. Individual colonies were picked into a 96 well plate containing 100 μl/well of LB broth supplemented with ampicillin and grown overnight at 37°C. Approximately 25 μl of 80% sterile glycerol was added to each well and the cultures stored at -80°C.

Preparation of plasmid DNA

Glycerol stocks were used to inoculate 5 ml of LB broth supplemented with 100 μg/ml ampicillin either manually or by using a Tecan Genesis RSP 150 robot (Tecan AG, Hombrechtikon, Switzerland) programmed to inoculate 96 tubes containing 5 ml broth from the 96 wells. The cultures were grown overnight at 37°C with shaking to provide aeration. Bacterial cells were pelleted by centrifugation , the supernatant decanted, and the cell pellet stored at -20°C. Plasmid DNA was prepared with a QIAGEN Bio Robot 9600 (QIAGEN, Chatsworth, CA) according to the Qiawell Ultra protocol. To test the frequency and size of inserts, plasmid DNA was digested with the restriction endonuclease Pvu II. The size of the restriction endonuclease products was examined by agarose gel electrophoresis with the average insert size being 1 to 2 kb.

DNA Sequence Analysis of Shotgun clones

DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS (Perkin Elmer, Norwalk, CT). DNA sequence analysis was performed with M13 forward and reverse primers. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). Approximately 4 sequencing reactions were performed per kb of DNA to be examined (384 sequencing reactions per each of nine PACs).

Assembly of DNA sequences

Phred/Phrap was used for DNA sequences assembly. This program was developed by Dr. Phil Green and licensed from the University of Washington

(Seattle, WA). Phred/Phrap consists of the following programs: Phred for base-calling, Phrap for sequence assembly, Crossmatch for sequence comparisons, Consed and Phrapview for visualization of data, Repeatmasker for screening repetitive sequences. Vector and *E. coli* DNA sequences were identified by Crossmatch and removed from the DNA sequence assembly process. DNA sequence assembly was on a SUN Enterprise 4000 server running a Solaris 2.51 operating system (Sun Microsystems Inc., Mountain View, CA) using default Phrap parameters. The sequence assemblies were further analyzed using Consed and Phrapview.

Genomic sequence of the KCNQ5 gene and its exon/intron organization

Genomic DNA sequence from PAC 141B1 was compared with GenBank database entries using the BLASTN and BLASTX algorithms of the AceDB package. This comparison originally revealed a total of 5 exons (exons 3(D), 4 (A), 5(B), 6(E), and 7(C) delineated in Figure1), based on their homology to the known potassium channel genes KCNQ1, KCNQ2, KCNQ3, and KCNQ4. Full-length cDNA was rescued from the pools of the human fetal brain cDNA library using the RCCA technique described in Example 2. Comparison of the cDNA sequence and genomic sequence of PAC141B1 revealed a total of 8 exons (exons 3-10 delineated in Figure1). Genomic regions corresponding to exons 1,2, and 11-14 were not present in PAC141B1.

In order to identify the genomic region corresponding to exon 2 and its right flanking intron, oligonucleotide KCN-2L2 (TTTTCTCCTTGTCTTTGGTTGCTTG; SEQ.ID.NO.:11) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2L1 (CCTCAAGTTGCCTCTTGATCCTG; SEQ.ID.NO.:13) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 2 and its left flanking intron, oligonucleotide KCN-2R1 (CAGGATCAAGAGGCAACTTGAGG; SEQ.ID.NO.:15) from the KCNQ5 cDNA in combination with the adaptor primer AP1

(CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2R2 (CCAATTTTGTGTGCTCAGGGATGGTAGA; SEQ.ID.NO.:16) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 11 and its right flanking intron, oligonucleotide KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.:17) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-11L2 (GATGATGTATATGATGAAAAAGGATG; SEQ.ID.NO.:18) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 11 and its left flanking intron, oligonucleotide KCN-11R1 (CTGATAGCTCGAATGACAGTTTT; SEQ.ID.NO.:19) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN11-R2 (AAGTGGTGGGGTGAGGTCTTCCACTG; SEQ.ID.NO.:20) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 12 and its right flanking intron, oligonucleotide KCN-12L1 (AGA ATT ATG AAA TTT CAT GTT GCA; SEQ.ID.NO.:21) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-12L2 (AAA CGG AAG TTT AAG GAA ACA TT; SEQ.ID.NO.:22) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 12 and its left flanking intron, oligonucleotide KCN-12R1 (ACG TGT TTG TTG GCT TTT AAT TC; SEQ.ID.NO.:23) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-12R2 (TAC ACA ACA TGT CCA GAT GAC; SEQ.ID.NO.:24) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 13 and its right flanking intron, oligonucleotide KCN-13L1 (TGATCAAATTCTTGGAAAAGGG; SEQ.ID.NO.:25) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-13L2 (TCACATCAGATAAGAAGAGCCGA; SEQ.ID.NO.:26) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 13 and its left flanking intron, oligonucleotide KCN-13R1 (GTTTTTCAACCTTGACCACCC; SEQ.ID.NO.:27) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-13R2 (AGCATACTGAGATCGTCTGTGGT; SEQ.ID.NO.:28) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 14 and its left flanking intron, oligonucleotide KCN-2543R(AATTCCAAAAGTGTCTGTCTCTGTC; SEQ.ID.NO.:29) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-

amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2512R (GGACCCACCTCTTCATCAGTTA; SEQ.ID.NO.:30) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

Products obtained from these PCR amplifications were analyzed using ABI 377 sequencers according to standard protocols. Comparison of the full-length KCNQ5 cDNA sequence with the sequences of PAC141B1and sequences obtained in PCR reactions with DNA from the GenomeWalker kit revealed all 14 exons of the KCNQ5 gene. Exact sequence of exon/intron boundaries within the KCNQ5 gene were determined for exons 2-14. The splice signals in all introns conform to published consensus sequences.

EXAMPLE 2

Cloning of KCNQ5 cDNA

The DNA sequence of the cDNA fragment that matches exons 3(D), 4 (A), 5(B), 6(E), and 7(C) of the KCNQ5 was deduced from the genomic sequence of PAC 141B1. Subsequent sequencing of PCR fragments obtained in RCCA reactions confirmed the presence of this fragment in the cDNA library from human fetal brain. This original cDNA fragment corresponds to the cDNA region with coordinates 368-1,004 in Figure 2.

A PCR based technique termed Reduced Complexity cDNA Analysis (RCCA) was used to extend this original cDNA fragment. RCCA is similar to procedures reported by Munroe et al., 1995, Proc. Natl. Acad. Sci. USA 92: 2209-2213 and Wilfinger et al., 1997, BioTechniques 22:481-486 and relies upon a PCR template that is a pool of approximately 20,000 cDNA clones; this reduces the complexity of the template and increases the probability of obtaining longer PCR extensions.

96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 483 bp PCR product using primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32). Eight wells were found to

contain the correct 483 bp fragment by PCR analysis. 5' and 3' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers , PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primers KCN-DL and KCN-ER because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primers KCN-EL (CTTTCTTTGCACTTCCTGCC; SEQ.ID.NO.37) and KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38). The PCR products were separated from the unincorporated dNTP's and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

PCR fragments were assembled into a contig termed "KCN consensus 2_16_99" that corresponds to the cDNA region with coordinates 278-1,456 in Figure 2. A second round of the RCCA analysis was performed to obtain the clones extending to the 3' end of the cDNA contig termed "KCN consensus 2_16_99". 96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 117 bp PCR product using primers KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.17) and KCN-11R1 (CTGATAGCTCGAATGACAGTTTT; SEQ.ID.NO.19) that were derived from the 3' sequence of the cDNA contig termed "KCN consensus 2_16_99". A number of wells were found to contain the correct 117 bp fragment by PCR analysis. 3' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.17) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer

KCN11-R2 (AAGTGGTGGGGTGAGGTCTTCCACTG; SEQ.ID.NO.20). The PCR products were separated from the unincorporated dNTPs and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

PCR fragments were assembled into a contig termed "KCN consensus 2_26_99" that corresponds to the cDNA region with coordinates 278-2,527 in Figure 2. A third round of RCCA analysis was performed to obtain the clones extending to the 5' end of the cDNA contig termed "KCN consensus 2_26_99". 96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 214 bp PCR product using primers KCN-2L2 (TTTTCTCCTTGTCTTTGGTTGCTTG; SEQ.ID.NO.11) and KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38) that were derived from the 5' sequence of the cDNA contig termed "KCN consensus 2_26_99". A number of wells were found to contain the correct 214 bp fragment by PCR analysis. 5' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer KCN-DR2 (CAGTCTTCCTTGCCATCCTC; SEQ.ID.NO.39). The PCR products were separated from the unincorporated dNTPs and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

PCR fragments were assembled into a contig termed "KCN consensus 3_3_99" that corresponds to the cDNA region with coordinates 1-2,527 in Figure 2. A fourth round of RCCA analysis was performed to obtain the clones extending to the 3' end of the cDNA contig termed "KCN consensus 3_3_99". 96 wells of a human

fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 145 bp PCR product using primers KCN-2106L (GCAGCCCCAACAACTTTACA; SEQ.ID.NO.40) and KCN-2250R (CATTTTCCTTGGAGGCAACA; SEQ.ID.NO.41) that were derived from the 3' sequence of the cDNA contig termed "KCN consensus 3_3_99". A number of wells were found to contain the correct 214 bp fragment by PCR analysis. 5' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers , PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-2106L (GCAGCCCCAACAACTTTACA; SEQ.ID.NO.40) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer KCN-2165L (GCCAGAAACTCTGCACCCTA; SEQ.ID.NO.42). The PCR products were separated from the unincorporated dNTP's and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols; PCR fragments were assembled into a contig termed "KCN consensus 3_15_99" that corresponds to the cDNA sequence depicted in Figure 2.

EXAMPLE 3

Analysis of expression of KCNQ5

RT-PCR: RT-PCR experiments were performed on "quick-clone" human cDNA samples available from Clontech, Palo Alto, CA. cDNA samples from heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and retina were amplified with primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32) in the following PCR conditions:

1.	94°C	10 min
2.	94°C	30 sec
3.	72°C	2 min (decrease this temperature by 1.1°C per cycle)
4.	72°C	2 min
5.	Go	to step 2 21 more times
6.	94°C	30 sec
7.	55°C	2 min
8.	72°C	2 min
9.	Go to step 6 19 more times	
10.	72°C	7 min
11.	4°C	

The KCNQ5 gene was found to be predominantly expressed in human retina and brain (Figure 3B).

Northern blot analysis: Northern blots containing poly(A+)-RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas were purchased from Clontech, Palo Alto, CA. Primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32) were used to amplify a PCR product of 483 bp from a quick-clone human retina cDNA available from Clontech, Palo Alto, CA. This fragment was purified on an agarose gel, the DNA extracted and used as a probe for Northern blot hybridization.

The probe was labeled by random priming with the Amersham Rediprime kit (Arlington Heights, IL) in the presence of 50-100 μCi of 3000 Ci/mmole [alpha ^{32}P]dCTP (Dupont/NEN, Boston, MA). Unincorporated nucleotides were removed with a ProbeQuant G-50 spin column (Pharmacia/Biotech, Piscataway, NJ). The radiolabeled probe at a concentration of greater than 1 x 10^6 cpm/ml in rapid hybridization buffer (Clontech, Palo Alto, CA) was incubated overnight at 65°C. The blots were washed by two 15 min incubations in 2X SSC, 0.1% SDS (prepared from 20X SSC and 20 % SDS stock solutions, Fisher, Pittsburgh, PA) at room temperature, followed by two 15 min incubations in 1X SSC, 0.1% SDS at room temperature, and two 30 min incubations in 0.1X SSC, 0.1% SDS

at 60°C. Autoradiography of the blots was done to visualize the bands that specifically hybridized to the radiolabeled probe.

The probe hybridized to an mRNA transcript that is predominately expressed in brain and retina (Figure 3A).

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An isolated DNA comprising nucleotides encoding a KCNQ5 protein.

2. The DNA of claim 1 comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:3.

3. The DNA of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1, SEQ.ID.NO.:2, and positions 138-2,675 of SEQ.ID.NO.:2.

4. An isolated DNA that hybridizes under stringent conditions to a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1 and SEQ.ID.NO.:2.

5. An expression vector comprising the DNA of claim 1.

6. A recombinant host cell comprising the DNA of claim 1.

7. An isolated KCNQ5 protein.

8. The KCNQ5 protein of claim 7 having the amino acid sequence SEQ.ID.NO.: 3.

9. The KCNQ5 protein of claim 8 containing a single amino acid substitution.

10. The KCNQ5 protein of claim 8 containing two or more amino acid substitutions where the amino acid substitutions do not occur in a position where the amino acid substituted in KCNQ5 is also present in the corresponding position of any one of KCNQ2, KCNQ3, or KCNQ4.

11. An antibody that binds specifically to a KCNQ5 protein where the KCNQ5 protein has the amino acid sequence SEQ.ID.NO.:3.

12. A method of diagnosing whether a patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration that comprises determining the DNA sequence of a region of the KCNQ5 gene from the patient and comparing that sequence to the sequence from the corresponding region of the KCNQ5 gene from a non-affected person, *i.e.*, a person who does not have Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration, where a difference in sequence between the DNA sequence of the KCNQ5 gene from the patient and the DNA sequence of the KCNQ5 gene from the non-affected person indicates that the patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

13. A method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

(a) providing a DNA sample from the patient;

(b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

(c) performing PCR on the DNA sample to produce a PCR fragment from the patient;

(d) determining the nucleotide sequence of the PCR fragment from the patient;

(e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

14. The method of claim 13 where the DNA sample is genomic DNA.

15. The method of claim 13 where the DNA sample is cDNA.

16. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of a sequence selected from the group consisting of: SEQ.ID.NO.:1 and SEQ.ID.NO.:2.

17. A method for determining whether a substance is an activator or an inhibitor of a KCNQ5 protein or a mutant KCNQ5 protein comprising:

(a) recombinantly expressing KCNQ5 protein or mutant KCNQ5 protein in a host cell;

(b) measuring the biological activity of KCNQ5 protein or mutant KCNQ5 protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein;

where a change in the biological activity of the KCNQ5 protein or the mutant KCNQ5 protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein.

18. A method of identifying inhibitors of KCNQ5 comprising:

(a) expressing KCNQ5 protein in *Xenopus* oocytes;

(b) changing the transmembrane potential of the oocytes in the presence and the absence of a substance suspected of being an inhibitor of KCNQ5;

(c) measuring membrane potassium currents following step (b);

where if the potassium membrane currents measured in step (c) are greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of KCNQ5.

19. A method of identifying activators of KCNQ5 comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an activator of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

20. A method of identifying inhibitors of KCNQ5 comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an inhibitor of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is less than the amount of FRET exhibited by the control cells, the substance is an inhibitor of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

21. A method of treating Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, age-related macular degeneration, other forms of macular degeneration, deafness, epilepsy, different forms of neuropsychiatric, heart, gastrointestinal, and muscle disorders by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a voltage-gated potassium channel containing the KCNQ5 protein.

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   1  CTGGAGTGAG GCGCGGGAAG ATGCCTGGTC CTTGCCTCGC GGACTTGGCA
  51  GCCGCGTCCT GCGGGTCTGT CCACTGAACT GCTGAGGACT GCGGCGGTGG
 101  CCTGAGGGAG AGCCGCCGGG GCAAGCAGGG GGCCCGGATG AGCCTGCTGG   Exon 1
 151  GGAAGCCGCT CTCTTACACG AGTAGCCAGA GCTGCCGGCG CAACGTCAAG
 201  TACCGGCGGG TGCAGAACTA CCTGTACAAC GTGCTGGAGA GACCCCGCGG
 251  CTGGGCGTTC ATCTACCACG CTTTCGTnnn nnnnnnnttc cttttctatt
 301  cttattatta atatatgatc ttattattaa taatataaag gaatagcaaa
 351  tgagaatcca tgagcaatat cagaccatga aaatgagcca gtggctgagt
 401  aacaaccaat taggacactt gatagtttag caaagttgcc aaacaggaga
 451  cagactcggc tcctttgaac gaagagtgac tgcagtgtgg attccccaga
 501  taggagagca agaacatact ttctgggcct ctctcaggat cgttgtttgg
 551  gaaggaagtt gtatgggaaa ttcacaaact cttagatgct aacatttaaa
 601  tgcagcatgc cacacacaca aacccacaaa cacaaccttt ttttcatcaa
 651  taaaattgca gaggagcccc atttgcacag tatatcacat tgtattttaa
 701  tatccaaaat ggctagtccc ttccagagtt tttatgagtt aatgtgtgct
 751  aatttaatgg gcctggtgct ttattcattt gaagcaagaa attaagtctg
 801  tgataataag gtaaggttct tatcagattt ctcttttttgt tgttttacag
 851  TTTTCTCCTT GTCTTTGGTT GCTTGATTTT GTCAGTGTTT TCTACCATCC   Exon 2
 901  CTGAGCACAC AAAATTGGCC TCAAGTTGCC TCTTGATCCT Ggtaagtgaa
 951  acatgaacaa gaacgtacat gaatgttgta taagaactgc ctataacatt
1001  tatactatgc atcttatcct acaaaaaaat cctatctaaa aaagagttac
1051  tgagaaatat aaaaatgtca aagattactg aaacatttgc ccaccaattt
1101  aacatgtagt caatccttag aaatatatag aaatgttcag gattgctatt
1151  acacagcaat atcttgtgtt gtagatatat cataaataga aggcaatatt
1201  agaaagcagt tttaagtatg tttatctatg ctaataaaca aattatataa
1251  gaagaatcag tatctatgag gcctctcatt atattgtgaa agactataga
1301  gtagagagca ttttccaata actgtaattt ggcagtagct aaatataatt
1351  ggccaagaac tatgaacata tggcacctca taagaaaata gaaggctcct
1401  tcatgctctt ttcaaccaac agactgcatt atgagttttg ctgctaatgc
1451  agttacctgg tgataaattc tgcagtttgc tctgtttcca ttatgctgtc
1501  aatcctcaac cacacagaat tgctcaattc actttnnnnn nnnnnacgag
1551  gtcaggaggt cgagaccatc ctgcctaaca cggtgaaacc ccgcctctac
1601  taaaaataca aaaaattagc tgggcgtagc tactcaggag gctgaggcag
1651  gagaatggcg tgaacctggg aggcggagct tgcagtgagc cgagattgcg
1701  tcactgcact ccagcgacag agccagactc cgtctcaaaa aaaaaaaaaa
1751  aaaaaaaaaa gagtatactt gatttatggc atgagtggtc ttgaatgatt
1801  ttgatggatg actggaaaca attagagata taaataaata gcacagaatc
1851  atgacagatt tcatgaagaa tacactgtga agattcacat ggttaataac
1901  attgaaatta ttaaaataaa agagactgca tatattagat ttttctttgt
1951  gatctagttg ttcaaagcag cagaaaactt taaattttcc ttaattttga
2001  aagtgtgatt aatggaatat tgttacaatg ccattgattt atatactttg
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FIG.1A

2051 aggatagtta acttctttat gtttattaga aattgcactg agagctaata
2101 tgcagtttct attggtggtg atatgttctg ttaacagggc ctcctgtcag
2151 tttttattct gagatttctg cctttcctgt tcttttgcat gagcctaact
2201 gactgagttt caatatcaag ttctgaaagc aagtgagcat actagtgtgc
2251 atgcaatgtg cacagaaaga tgtgttttcc tacctctcaa agctcaccaa
2301 aggatattac tcatattgcc acaaagtaca tttacaccaa taacacatac
2351 tttggtaatg ggaaaaataa ataatcttag gtaataaaat gcactttggt
2401 gcttataaag gaaaataatc atcacaggta gaagaggagg aaggcaagac
2451 taggttttga ttcaaaatgt tttctgtttt gctaggaagt ctagaaggaa
2501 tttggtcaaa tgacctgagg aggcaaggag atttctccca gtggcacatg
2551 gcagtccaca agaagactag gggcagggaa acaaagcaag attgtatctg
2601 atttctacag gactccaaca ctaccctgcc aataccctttt tctcctctga
2651 aagccatgat cccaaaggtt tccatttctt ccgggttttt cacattctac
2701 caacaagcag aatgtcttcc ccactgacag ctcaacagtt gagtttttcg
2751 gggttctacc ccttcatcaa cacagccttt gctttcctgg ttactgagta
2801 aataaaacac actggccact tctgcttagc cttcaggagc cactctggct
2851 ccaccctgaa gttccatttc ctccaagaac taagacattt ggagattctg
2901 cctaaaatat gcttaaataa aagggataaa ctgtctctca ctttctttcc
2951 cccacagctt gtcttaagaa caacggtgta accattagtg aaaaatcctt
3001 tcaggctagt aaaatgtaaa aagagaagag gtatcgggta acaacaaatg
3051 aaaataaaat cattcagtgg tagaaaccat aggagtgaaa ttaggtagtt
3101 actgtcctcc agcttacatt aatcaaaata agcccacact gtcactaaga
3151 catcttgcag taagaagcca ttcaccctct cattgcaatt agtgtcataa
3201 agctgaatag acaggtcaga aatgaaaatc ataggccaat taagttattc
3251 tcatttttcc acctccagct agctcttttt gagaattttg attttaatag
3301 ataataaaac agtatgctaa ttgggtggtg ctgagcctga agccgtgaac
3351 tgaaggaaac ttcaacaaac atttggctag ggcctgctct aagcagaggg
3401 gctacgacaa tgggtaaggc atttctctag aaaccagcac tcagcagaat
3451 ttctggcacg agattttgtg agttttctag tggtgtgtct tatcttcccc
3501 caatagatta taaactcctc gagggtgaca gaacacattg ctgatcttta
3551 tatcctgcat gtttatggta ttcggtagac atttataaaa tgaattcatg
3601 aaaagcagaa catctagaaa tgaagtcaac attgcagaga agttagctat
3651 atgaaactct gtaaataccc tcaaggaaga aggaagaagc agattttctg
3701 gaaatcaagg gacctcccag tataagagca attcctttgg tccaaagaag
3751 ggcagctata agaacagaga cataactcct ccgtaagaaa agataaaaat
3801 tctgaaagtt ctctaagaac atgcccagcc ctgacctgcc tctcactctc
3851 agtagcatgg aagccgtact atctcttatc ttgacacaag catccacttc
3901 aatccacagg acaaatatca ccaatatgag aagataagat ctgttttata
3951 gtcaacttgg ctgacatgta ctaatttctc aaggtcagga tagctctcta
4001 aatagaaata cttgtttgtt ttaaggaatt ttatttaaat ttgtgtatct
4051 taaattttaa ttttgatctc ggctcactgc aacctccacc tcccaggttc

FIG.1B

4101 aagcaattct cctgcctcag cctcctgagt agctgggatt acaggcacct
4151 gtcaccatgc ctggccaatt tttgtatttt tagtagagac cggatttcac
4201 catgttaacc aggctggtct caaactcctg acctcaagtg atctgcttac
4251 caaggtctcc cagagtgctg agattacggg catgaaccac tgctcccagc
4301 ctcatgtagt tcttaagagg aagaaaagcc tatagattag tgagaagtag
4351 acaaattagc aatttgaatc aaatgaaaac ttggtttgat ttcattcatt
4401 ttggagacac tttcggtgtt tccattttga tctgattcag gacactgatc
4451 ttcgattcta agttgcactg gttaatttgt gattatttta caaaatcata
4501 acagaataca tatctgggtc cagttcaagg tacagcaagc cattttcaat
4551 gtttccagct tggtgtttat gattcataca tgaatcattt gggcatgcag
4601 acacacccaa atagaaacct caaaccaatt aaggtataaa taggtttagg
4651 atgggggagg atggacaaca aaaaaaacct cagtaaggct cctccacaaa
4701 gggcaccact tcagcttggt cccatgggca gacttcacct gtggcaggtg
4751 aaggggaaag cctaagacat cctgtgccct gatgcagatg tgacttacag
4801 aacataaaac gtaaaggcag aagggatcat ctgggtggct gctcatacta
4851 ggcaggcatt atagctgctg acaagagcca acccatccct aaggatctaa
4901 aatcactaca agagaggacg gaagtcctct catcatggca tcccaactag
4951 taactgggat gcatagggcc actccaggta ggcagcgagg ggtaaaccag
5001 gaatgtcctg ctgttcccac ctcttttccc caagcagggg gcacccactt
5051 cctgcctcca gcccatatgg tctcaattta tggcctcaat acctgataaa
5101 aaaccagccg tgatccttcc ttagtaacaa agtgtcaatc agtcatttgg
5151 aatggggcat tttcaaacta ttgcctccct atgatcttct gaagaacctc
5201 tgggataaat gaacattgtt atttaggtat ggttaaatga attacttttc
5251 aggagcagat ttatatatta acaggtgtga aaaacttgaa tggataaatc
5301 atttataaag aaatttcagg aatcaactaa tcccaagggg aaagacccac
5351 taagacacta gattttgctg gagctactca aacaaattca tctatcatat
5401 ctacctaagc cttccaatca atgagactag ccgcagtgac actgtctgtc
5451 aaaactacct cagtcatttt tttccaaggc aggaagcatc catacccctt
5501 cacaccttaa tctaatttcc cctatctcca ccccactccc acttctatga
5551 ccccccttccc cccataccca cccccgaagc atctactagc caacttagtg
5601 aagttctgtc acgctctcac aaccacctct gtgcagcaat gattctgtaa
5651 atatccatgt gtcctcaaca ccaggtcaaa ttagtccctt tggtaaaaac
5701 attcattccc tcaaattctc ttccaataca ctaatatacc ttcccaaaaa
5751 gtaaggagaa gcttgaaagc tagctggatt gatgatggta tgtgatgttc
5801 tataagttat agttagtaag catgttttta ggatattttt ctgctctcca
5851 aagagacaca attccggaag atatttactt ttgtgtattt ccacattttg
5901 gtttaagttt ggagccatct ctagatctta atttcattcc cctaatatgt
5951 gttatactag tagaattttc caaattacat agaattataa ctgcaactct
6001 tctgactgat gccttttttt tgcattttat gatgcagttt acatcacaaa
6051 ttctttccct gcagggatta tgtaaagagg catgttgacc tgctagccct
6101 atgttacttt aagtatatgc acacacacaa aaaggaacaa aaacagctgg

FIG.1C

6151 gaattgatta tgttgatatt ctgaataaaa gcaatagttc taattatgta
6201 tgtctaatta gccacagctc ttcaagaatt gctgcaaatg tcacagggtt
6251 tataatgtcg gcatttcatc ttcacgaaaa tgctatttga tggcataaaa
6301 ccagaaaaaa ctaatggtca cagaagacag cttgtagatt agacaaaggt
6351 cactgtgttt taatgaacag tgctgttaat taatgagaaa acaactggta
6401 catgagcttt taagcattgt gaatttgtac ccaaaaaatc aatctgccta
6451 aaacaatttt aagtagctaa aaaacaaaat aacggcaaga acataattta
6501 aacctcaaat ggtacagcag agttatatgt atcaattaaa ttgaatcaca
6551 gttctcaggt gtgacatatg aagaggcttc tttaatgcct ggaaaagagg
6601 gttaatatgg attggtattc tcaatacata ttgtagataa aattcaagac
6651 tagctctacc actgcctctt ttctttttt ttttttttt ttatttgaga
6701 cagtatctcg ctctgttgcc caagctggag tgcagtggca tgatctcggc
6751 tcactgcaag ctccgcctcc cgggttcacg ccattctcct gcctcagcct
6801 cctgagtagc tgggactaca ggcgcccgcc accacaccca gctaattttt
6851 tgtattttta gtagagacgg ggtttcacct tattagccag gatggtctcg
6901 atctcctgac ctcgtgaccc acccgcctcg gcttccgaaa gcgctgggat
6951 tacaggtgtg aaccaccgcg cccggccacc actgcctctt aggcttctta
7001 atttccttat catttaagaa gaataagaaa atgcttctat gttttaccaa
7051 aattctgtga ggacaaatga ggaaccattg taactcctac aaggtgagtg
7101 ataaaaataa tacacattta ttgtctttgc ttttgtaaag agttatccaa
7151 gccaagcttc taggggctta aataaggaag gacaggacca ttgttaataa
7201 catcaagttt ccactacagc tttcctccaa acaagtcaaa tattctgaat
7251 attattcact aatctcttta gctgccattt cagtaaatag cgagcatttt
7301 atttcaacta aaaccaagca agagaaaatg aactgcttta tcctgaggta
7351 cagcagcaaa ggcaccagaa cttgtctcat ggcttaccca gcaagggtca
7401 gaagaaccat ccctaattta aatcatctcg actgaatgta acagattttt
7451 gtattttcaa ctcatatgaa aataaaacaa tgagacctca tccaaagggt
7501 gatttagaga gtacctctaa acaaaacaca gtgaaaatag acccagcatc
7551 tggatttgaa gaacacattt cctcttacga gtctatccca ttgtctagat
7601 tgctggcaat ggctttttta aatttaaatt gttattgaga taattgtaga
7651 ttcacatgca gttgtaagat atagtagaca taccctgtgt atactttacc
7701 caatttccgc aaaaggtaac attttgtaaa actatagtat aatatcacaa
7751 ccaggataat aatattgata cagctcacaa atctcattca gatttctcca
7801 gttttacttg aatacatttg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg
7851 tgtgtattgg ctctgtacag ttttatcact tgcagagatt agtatatcta
7901 aaacaacaat caagatattg gacagttccg gccgggcacg gtggctcact
7951 cctgtaatcc cagcactttg ggaggccgag gcaggcagat cacgaggtca
8001 ggagatcaag accatcctgg ctaacacggt gaaaccacgt ctctactaaa
8051 aatgcaaaaa attagctggg cgtggtggtg ggcgcctgta gtcccagcta
8101 ctcgggaggc tgaggcaggc gaatggcatt aaccagggag gtggagcttg
8151 cagtgagccg agatcgcacc actgcactcc attctgggca acagcgcgag

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FIG.1D

8201 actctgtctc aaaaaaaaaa aaaaaaaaaa gatatcggac agttccattg
8251 tcacaaggat ctctcaagtt acccccttgct aaccacatcc accttctcct
8301 tcacacttgc acacccccttc ccttctccag cctgacccca gcagccacta
8351 atctgttctc catttctgaa atgtttttat ttcaaaaatg ttatataaat
8401 ggaataatac agtgtataac tttttaagac tgacttttct tgcactcaat
8451 ataattccct ggcaattcat ttatgttact ctgtgtatca atagttcatt
8501 catttttatt attgagtagc attccatggt atggaggcac cagagcttgt
8551 ttaaccatcc tcatggtgaa ggacatctgg gctgtttttg gggtctggtc
8601 attatgaata cttcttctgt gaacattcat gtacaggttt ttgtacaaac
8651 ataagtttc atgtctctga cacgaatgcc caagagtaca attgctgagt
8701 catatggtaa ctatatgtcc agttttataa gaaacgacca tgctcagaag
8751 gaccatactg ttttacattc ccatcagcag tgtttgaatg atccagcttc
8801 tccgcatcct ccccagcatt ttgtgttgtc actatttttt gccactattt
8851 tttattttag ccattctgct agctgtgtag tgataccatt gcgatctaat
8901 ttgcattttt ctgatggcta atgatgtcaa ataacttttc atgtgcttct
8951 ttgccatatg tgtaacttct tttatgtatg tcctctttta tgaatatcct
9001 atttatacct ttgccaattt tctaattgag tttttggttt tttactgttg
9051 agttttaagg tttctttaca tattttagat attagtcctt tgtcagatat
9101 gtggtttaca aacattttct cccagtctat ggcttgtctt ttcatcctta
9151 gtacctgggc tctcacagag taagttttta cttttgatgg agtccaattt
9201 ctcatttttt ccttttataa cttctgcttt tgatgtcaag attaagaact
9251 ctttgcttag tccaaatccc aaaaatatct ccatttttt cctaaaagtt
9301 ttattatttt atgtttaatt tttaaacccg tggtccattt ttaaatgatt
9351 atcataagat aggaagtata gattaaggtc cactttttg cctatagatg
9401 tccaattttt ccagcatcat ttgttgaggg cccttcttcc tccattgaaa
9451 tgcttttgca cctttaaaac aaatcaattg agcatatttg tgtgggtcta
9501 gttctgaatt ctctattctg ttcactgttc tatatgtctg tatgttccat
9551 atgtctatcc ctccaccaat ccacagtctt gattactgga gctataatag
9601 taggctttaa tatcaggtaa agtgattgat ctcgttttat tcttctttgt
9651 cagcattgtt ttagctattc caggtcctgt gcatttccat ataaatttta
9701 aaataagttt gtatctaccc cccaaaaaaa actttgctga gattttgaca
9751 ggaattttat tagacctgta gatcaatttg ggaagaattg acatctttgc
9801 tatgatcagt cttccaattc ataaacacgg tatttctcct cacttattta
9851 ggtcttttct gatttctttt atctgcattt cataatctca atgcacataa
9901 accgtatgtg tttttctaag tggatattta agttatttt atttggaatt
9951 attataaata ataattttgt gtttctaatt ttcatttcca catgatcatt
10001 gttagtataa agaaatatga ttaatttta tattgatcat acatcgtgca
10051 gccttagtga actcacttgt ttcaagagtg tcttcgtaga tttcttggga
10101 tttttctgta gaccatcatg ccatttgtaa atagagacca ttttatgttt
10151 acctttccaa actgcatggt tttttttctt ttatttggct tattgcagca
10201 actattaata taacttacaa tactatgttg aataagagtg atgaaagtat

FIG. 1E

10251 acatcttggt tgttcttaat gttggggaaa agcattcagt ctttcactat
10301 taagtatgat gttaagtttg ttgtaaatgc tatttatcga attaaactgt
10351 taaacctctt ttcctaactt gccaagagtt ttttttaatc ataaatgggt
10401 gttggatttt gtcaaatgct atttcgtcat caatttatgt gatttttttt
10451 tagctttatt atataatgca ttatattgat tgatttcaac cattgagcta
10501 gccttgcata cctggaataa atcctacttg cctatatatt gactgattga
10551 ctgtttgcta atattttgct gaggattttt tgtgtctaag tttatgataa
10601 atatgggtat gtagtttttg ttttgctttg ttttattgtc tttgtctggt
10651 tttggtgtca ggataatact gacttcataa aatcattgag aaagtttttt
10701 ctcctctatt ttctgggaga aattgtgtga aaatgatata ttcttattta
10751 aatgtctggt tgattttgcc agtgaaacca tttgacctgg acatttctgt
10801 ttggggagat tttaaactat gagttcaatt gctttagcgg ttatagggct
10851 acctagattg tctatttctt ctttgttgag ttttggtagc ttgtagtttt
10901 aaaggatttg gtccatttct tctaagttat caagtgtatg tgcgtaaagt
10951 taccatagta ctccttcatt ttttcagtgg cgtagaatct atactgatat
11001 cccctattac attcctgaca ttggggattt tcatcagctt tcaagaaaga
11051 gatttatcaa ctttattgat tttctcaagg aaccactttt ttgtttcatg
11101 gattttccat attgttttcc tcttttcagt ttcattaatt tttgctcttt
11151 tctatgttat ttccttctat ctgcttcctt tgggtttatt ttacccttct
11201 ttttccaatt tcttgaggta ggtactcaga ttattaatat gagaactttc
11251 ttcatctaca atgtaagcat ttagtgctat aaattttcct ctcagcactg
11301 cttttactgc atcctacata ttttgttatg tcatttttt attttcattc
11351 agttcaattt ttttaaaatt tttgagactt tctctttgac caatggacta
11401 ctgggaatat tgtttaattt ccacatgttt agagatttgc ttgttgtctt
11451 tctgttattg atttctagtt ttattccatt gtggcttgag aacacattct
11501 gtctgatttt attcttttaa acttgttgaa atttgtttat gacccggaat
11551 ataatctatc ttaataaatg tgcatgggtg ctttttaaaa atgtgtattc
11601 tgctgatgtt gggtgaaatg tctcatatca gtcagatcct gcaggttgaa
11651 tgtgttgttc aaatagttca catacttgtt aattttcatt ctagtagtgc
11701 tatgcccata gttctgagca aggataaggt gggttgatga agttcccaaa
11751 tacgacttaa catttgtcta tttttccttt cagccctatt ggttttgctt
11801 tatgaatttt gagactgtct tgtcattttc ccctcacctt cttaaaaaaa
11851 aaagcagcaa gcaatatttc tgaacaatag ggcttgaata aagaacttgt
11901 aaatgtactt tatcctgcct tactgcagca gaggaatagt gcagtgacct
11951 tggcatacct aagaacaatg ggcatatatc tgtcatttgt taagaacaat
12001 ggacatattg gtgtcatctg tttccatgaa aaagaaaaat tactacacag
12051 tagtgttcgg gggctcaggc cacatttggg attttcttgg gatcccttat
12101 aatatgagta ctcacaactg gagtgccaaa tgtcctggca taagcaggaa
12151 ttaggggcag ggattaaaaa gtggatctgc cattcaacct aggttgggca
12201 caggcacaac tacatgggca ttttgggcct gagtatgttt tccatatttg
12251 gaaagatgtg ctggattaac aagaaatgat ctggcacttt agatacctta

FIG.1F

12301 ataaatgaac agactcaact acccagatat acattcaaaa ccaaaacaaa
12351 gccaaaatca aacaaaacct tcggattatt aatgaatgta tgttgaccct
12401 aattttggtt atctgtgcat aaagtcatct ttgaaggttt atcctataag
12451 taattttgaa taactgtgag taagcagtga tgatattggg tatttatttc
12501 ccatcatgag aaatatttgt ggattactta gtgcaaaata agagaaggat
12551 agaatgatat agctctacta ggcatttcaa aagtgcagca tatcatcaca
12601 tgctctaatt aaagaaacag acatttgcct gaaatttgca acaaaaatgc
12651 cacttttata aatttggtaa cagtttttat cctgcgagta agaatgggag
12701 aatggggagt aggttagaat gtttactttc tctgctttgt atttctgtga
12751 tgtttggaag ttttaataaa gacaatatat tacttttaca attaagctat
12801 tataacaaca atctataaat attgttctac attataaatc ttgaatcaac
12851 cctcgtttta aaacaagaaa gtgaattatt ttttaaagga aaaacaattt
12901 tgataaataa agtgcctgtc cctatagaga cagcaaggtg actgtgctaa
12951 gcttgctcca gaaatgcttt cgttttacaa attccatttg gtttttcatt
13001 ctatcatcga ctgtttaatg agccactcta gctggccttt ccccagggtt
13051 aactcacaac tcttcctaaa taatctcagt cattttcctg gagcaggtgt
13101 tgttttctag gcagctcttg ccaaagggca gcataataat taacaagctt
13151 cagatctgga ggattcaggg tctcatttct ggcaaaggat atgtgtgtca
13201 cactgtaatg cttttgaaac ttactgtttt ataaggagat tttgacactg
13251 taacgaaaga attcagtata cttcgaaata tttgcttctt tttctctaag
13301 aacaactctc gaagatttag agattatgtc actccttgca gcaaaatgaa
13351 gctctatttt ctgtaaaact tgccattata tttcaggatc gctattttat
13401 ttcagatatt tcacatgtta ctgggtagct aaagtcagct acactataaa
13451 ttccagacga gacagatcca ttattcagcc tgcacattta gtattgaaac
13501 tgactcccaa atttacgaag cattaatgac ttgaaaagga atagcattcc
13551 atgataggaa atgcagacag tatgaggttc aaatgctgtc cagatttagt
13601 ttgatctgga gaaaggaaat gttcttaact ttagatatta acaactagat
13651 cctttagaca aagtgcctaa atgtccaaaa atatgcatag agcttcttac
13701 tgtggtttgc tccataatgc ttctctctga tatgtcttat ctgatatttt
13751 agGAGTTCGT GATGATTGTC GTCTTTGGTT TGGAGTTCAT CATTCGAATC Exon 3(D)
13801 TGGTCTGCGG GTTGCTGTTG TCGATATAGA GGATGGCAAG GAAGACTGAG
13851 GTTTGCTCGA AAGCCCTTCT GTGTTATAGg tgaatatcag agtctcagat
13901 acctggacta tgacaccaac attcttgtct tctatctcct gttttgcaat
13951 atttgattaa tattaaaagc taacatccat ggagtactta tcatggaccg
14001 ggcactcttg tgtcttgaaa atgttgactc atttgatgtt gtctccacag
14051 cattatgagg tagatatttt tattatcccc attttacagt tgaagaattt
14101 gagacaaaga gagacggagt tagttgctga atgtcataca gctactaagt
14151 ggtagagctt gaattttagc caggcaatct ggctctagaa tacccactta
14201 tatcaattaa cttatatgga aatacatgtt tctccagtgt tgccctaatt
14251 taggggaaca ttgatttcag tgagtataaa aaacccttttt tgctctacaa
14301 tatgacacca gtttatgctc tggatcagct ggaatagata gccatacatt

FIG.1G

14351 ggtactggta atgcatatca attaacattc ttcaagaatg attataagta
14401 tggtttgctt aatgcattgg taactgtaga ctggacaaag tgctctacac
14451 tctgaaatat gaatccccca ccctccaatc caaaatаccc aaatacatag
14501 cacatctctg cacctcccta gctgccattg tttctgagag acatcattca
14551 cctgggttct agcttgaaat tgtacttcct aatgaagatg ccatagagca
14601 ttctggaatt aagatctagc atttcccagt aatatttaaa agtattataa
14651 aatggaaaag tctatatata aaagctatta ttattattat ttagtagcta
14701 aatttggctc tcttgctttt ttgtctttct cacttcctag gcagccattc
14751 aaatccaaat actgaaaatt cttgatgagg cttatttatt tcaactattt
14801 tcaacctgaa aaagtaggtt tttaatttac aaaatagttt aggctatctt
14851 ctacagctac cacaggtttt ctgttttgtt ttaggagtca ggaattgttg
14901 cgtaatcttc tcagtttcct cccttactcc tgttccctat ggtgggggca
14951 gagtggattt aaggaagaaa gtgtatcacc atccatgggc actgttaaga
15001 agaatccttt attccatact tctttcatag tatgcacatc tcaaaagtct
15051 ccacttatta aaccagaatt tatggaaatg tggctgtgtg tgaggcactg
15101 gtgttacaat agtgaatcta gtggaattct cagttaaatc tatcttaaaa
15151 taccttgttt ggccctcacg aatgcagtct gtttgtctgt cttctccaat
15201 aataggcttt actgcttttt ggaagccatc atgctaaggt atggagcaaa
15251 gtcggtggta actccaagcc ttgcaggttc ataaagcccc aaggcatatt
15301 agtgaaggca ttagattttt ctccctggag atgcttttt ctctctgaat
15351 tctctactca ggcagcatta caaaggactg taacctgatg tgaaactact
15401 gcacaacaag tactttctgt cccataaggg gcccaaacaa aacaattcac
15451 aaggcagatg gttagccaag attcactcaa cttctacagg ataaggtcag
15501 ttcatggatg actggaaaaa tgtttcattc cagattttaa aaccaattaa
15551 aaattggttt tgttccaatt tcagaagcaa tgccaatgta tctctggcct
15601 ccatgcgaaa aagaaagctt tttcccagat gtctgctgcc ttttgatagc
15651 tgttttcagc tacggataca tcaacacatc ttagtccttt agtctttctc
15701 ttcctgcctc agatccttgc aggtcttgat ataatatgta gttctaaaaa
15751 tgatgttatg ttatctataa tcttacttgc ctttggatgt atcaatagtg
15801 accttaaaaa tttagtatga tgcctggcgc agtcactcct gcctgtaatc
15851 ccagcccttt ggaaggctga ggaaggcaga tcacttgagc ccaggagttt
15901 aagaccagcc tgggatggaa agacctgtct ctacaaaaat ttaaaaatta
15951 gctgggcatg gtggcataca cctgtagtcc cagctactca gaaggctgag
16001 actggaggat catttgagcc caggagtttg aggctgcagt gagccatgtt
16051 cctgccacta cactccagcc tgagtaaaaa gagtaagacc ctgtctcaaa
16101 aaaatactag tatgacatgg tttacgcata attgcagttt tgttaaccct
16151 tcagaccttt tctttattta gatattttac ctctaggaaa ttaattgctt
16201 tggaagaaaa tttcatggat aaatatataa tgaatcccca ttagaacaaa
16251 gtctagattt gtacttacag ttaggataat tatgaatttt ttagtctttt
16301 attaaatgta catgtttgac ccatggtcca gctgaatgga tgttatagtg
16351 aagcatgttc aaatgtggct tatcagtatg aggtcagtaa aatttaacct

FIG.1H

16401 acacaaagga atcaaacatc accttcctga aggcagcaag ctgtaccaga
16451 tgactgttga gactatttct aggcccgttc aacaggttca taaaaacgct
16501 tgatgtaact ggtaaaatac taaagtgaac actgtagtcg ccttgttcaa
16551 cctaatttac tgtgacaaaa tgaaaaaaga aatagcatct cttatctgtc
16601 cctgataact gttctgaatg ggtgtttgga aatatagcta aaacctccca
16651 aaacttcatg tatttgtaag atttgattac tctgaacatt ccaagtacac
16701 acccaaatat ttttagaagt gttgagcatt cgagaaaaga acatagaaaa
16751 gattgtggta gactggctgt gccttcaacc tgggaaatgc tttctgtaag
16801 cacaccaatg tttttctttc cagttggaaa tacactgaag ctgctagagt
16851 ggtcagctaa caagccccat gtactattgc accctattga caaaggccca
16901 attgatagag gttttatggg ctaattactg aaggttttag catgtcccct
16951 tctgaactgc ccaccttcct taccccctgca ctctagccaa aaccaagagc
17001 atttcaaaag cccactgatc tcacaaatcc taaaatcatt acatatttgt
17051 gtttggaaaa catattttaa acaattataa cataagggaa gaaataaatc
17101 tatttaactt tttttaatta ggtactttgt ttcattacta ctgtacattg
17151 atgtatgtct gcttggtttt aagcatttgc tttgaaaatt agtaaaatct
17201 tatctagaac ataaaattct tcctcatatc tatgtgttca atttttatct
17251 ctcccctaag actcttctgt cataataaca ttctatagct atgagcacta
17301 attgctgtat tgctgatgtg gatagtcaag tgtgttttgg gatgaccacc
17351 caagctgccc ttcatttacc cagactggct gctttatgat ttctctatgt
17401 gaaaacacaa aacactcacc caataacatc ggcataaaga atttgtgaat
17451 aacctctaag actgatttca tagtcattct tttttctagc tttcattact
17501 gacctaccca aaaattgcaa atagacctaa ggctgaaact tggatcacaa
17551 taatgagcct gatgtgtcag gaagccaaca aattacttct cacagtggct
17601 ttttttagat tccttggtga cgctttccaa aggcagatat aacagggcca
17651 tatttttcag tcttgcaggg agtctcagtg agaaaatacc agcctgttgt
17701 tagtcttgga tctgcatttg atgctaagtg taagttgatt tcttattgtt
17751 attttaacag tttatttttca gtgtaattat ttccacaaga ggacaaaaga
17801 ggaataaaat aaaatttttat tttatcctac gtggataaaa taaaattgaa
17851 gcctttatta aaatcgacca catggctgag actactctaa tatatctatg
17901 atttcatctt attaaaaggc aacttaagtt gctaatgtgt tcaaaagtta
17951 gatcagactc ccaaaactgc cactgtgttc taaattgatt ttgagacatc
18001 atttttctgt ttctctgtct tctctatctt ttgttctaag agacttactc
18051 ttcaatggca ttatttttcta taccttctac tccctttttcc ctttagtaga
18101 aattatatta tttcacttat tttctgttag tcatacactc ttctgcaact
18151 tattacttct aagtgttcaa actccctcag tgcatgtaga ttccttccat
18201 gtgattaact ggaccatctg aggacaatta aaacctgttg gattggctga
18251 tcacaaaaca ttaaaacaat tttaatccct ctagttagta atagctgata
18301 tttattgaga actactatat attaggtact actttaagca ctttataaat
18351 atggtcttat ttaattctac ataagtacta taatgtcctt atttatttta
18401 ttttatttta ctttacttta ttttattcta ttttatttta ttttatttta

FIG. 1I

18451 ttttatttta ttttatttta ttttatttta ttttttgagg cagagtttcc
18501 ctctatcacc caggctggag tccaaatggc atagtctcag ctcactacaa
18551 cctccgcctc ctgggttcaa gccattctca tgcctcagcc tcccgagtag
18601 ctgggactac aggcacacgc caccacaccc agctaatttt tgcattttta
18651 gtagagatag gatttcaccg tgttggccag gctggtcttg aactcctggc
18701 ctcaagtaat ccacccgcct cagcgtccca aagtgctggt attacaagtg
18751 tgagccacca cgcctggcct ttataatgtc ccattttata gatgaggaag
18801 taagacaaga ctgaagactt tatagatgag gaagcaagac aaacttaaaa
18851 agatgatagg tgaaagaacc tggatttatt tctcatgatt tcactctaga
18901 aaaatataca tacccaactg gaaaattgtc tttactgttt attagcattt
18951 taaaattaaa tttaattgaa ccttatctat tgtcatcaac ttgtaaacag
19001 aagaacatga tactattatg cttatcatta gtaattcaag atctttaaag
19051 aaaagccagg ctttatagaa cattaattgc agtatcagcc ttaattatat
19101 caactaaatt atgttaccta ttgctgctct ctaacctgaa tgggaaatta
19151 ggacctaaaa tagacttact tacctgtatc aaacttgcat atactcttca
19201 gttgttgtta tcatgaggct ccctgtgtat ttccaaattg gagacctctg
19251 gagagcaagt aacattactc gcaggactac cttcaaatgt tttttaacat
19301 taataaacta tcggtaattt agataattgt tgacctggta cacaaacttg
19351 aaagttgaac ttacaagaca gcttgattag ctgatatggt tggaccacat
19401 gaagaaaatt tatttccgaa gaggaaaatg aataaggatt atttcccact
19451 ctttctataa actttagtag agcactatga tatactgtat taacattctg
19501 tactatcaca tgttacataa gaaaattcat tcacagacag ggtatttatt
19551 atgtgtcaaa cataatctta agtataaagg atacaaaagc gactaaaaca
19601 cggtccctgc ctgtaagaat agaatctcct gtgcccttag agaatggcaa
19651 aacttaaagg aggtatacta taattatggt gttacagagc aaaggtgatg
19701 gaaattgaag tagtcctaga aagaaggcta tggaatatca tgagtgattg
19751 tataaaacaa agaagcaaag gactaccccc aggttttcaa attatgtttg
19801 agtcatccac tgaacattta tacaatttct accatgtcca agcatgctta
19851 tggatgctgg agaggctgta tggcaccaaa tgggcaaggt cttccttctt
19901 atagagataa tattcgtgtg gacaaagaaa aacaatacac aaatatgttt
19951 ttgaaaaaca agataatgtc atctacagat aagcacaatg aagaaaataa
20001 aacagaaaga gtagaggcta ccttagcttg ggtggtctga gaaagtcttt
20051 ctaagtagtt gacacttgag ttgacctgaa tattaagaag aagccagcca
20101 tgaggaggtt tagacacaga tctttccagg ccagggaata gtaattgcaa
20151 ggattattaa cacttgatga gcttgggttg tacaaggtgg agacataagg
20201 tctcgtggct ggagctcaat gagcagtggg gagaatagca gaaggtgata
20251 ggggagacag gcagaggtca caccctgaag gccatgtagg ctctgctacc
20301 ctgaatcaag gttccttcta aagacaggcc accaggcaaa ggtcctacgg
20351 gttgctggca ggcctaatat gaatactatt caggttatga acagcagcat
20401 taatcaggca acaaaaagta tggctgttta atgccctggg ttttttagtc
20451 atgtatgcct gttggaactt catatcagca atatacaaat tattacccat

FIG. 1J

20501 ttatgtttat aaaaaacata aacataagtt tataaggaaa taatcctctg
20551 aattttgttt ctatatttct gcccttagaa acatgaaatt ttacattgtt
20601 ttttaaaatg ttatttttct atgaggaact ggaattagaa tgtgatggaa
20651 actttttac agccccagtg gtatcaccag taagcagttc ctaagatcct
20701 ccttgccaag ggactctatt ccatactctt agttatgatg attgcagtgc
20751 agaattattt cattaaagat tgtttatttc ttttgagaaa ttgtcccttt
20801 ttgccttgag atgttatctg aaactgtatg atcaataaga agcattaaac
20851 tgtcactatg gggtaaattt tcaaagtggt gcctgcaatt tgccagtatg
20901 agtcccatta acttctgtgg aagtcctgca gttaaacccc tgcacatggc
20951 tttgaaaatg ttctacaagt ttgtctttgt ggtgcctttt ttatttttaa
21001 taaatatcac agagcaactc agtgcatagt tgattgcagt atttaagaac
21051 gtggataatg tactgtgcag gaggagactt ttaaaattag attttaaagc
21101 ttcatttcta ttaagagtca caacaacaat ataaatacat ctttagttct
21151 tcaaagagca cctttctata aaggaatttg gagctcaatt agaatataga
21201 gcttataaag cagcaccatg tccctctgag acattgttta cttcactcta
21251 tagatggaaa agtcgaggca ctgtgaagtg ggggtgcttt gtccttagcc
21301 cataaatcag cagtaaagct aggaatagca tgctggcctc ccaatagctc
21351 attgctctaa agcactgcct cttgttagag tcaaatttaa ttatagtata
21401 atccgtttaa tctacatgtg gagaaataat gaaggagcag gaaaaatgaa
21451 tttcttaaca gatcagtgga aaaccatatt acatagatta gcaactgtct
21501 tttcaagatc cgtattttcg gcatgtacta gatggtatct gatgccatga
21551 aagcaaatat tctaattcca ctggccagaa ctggcaaaga acttcttatt
21601 ttcctcactt tgaaacataa gtaaaggggt tccacttgaa aattaataag
21651 ccatgtgata tgattcgatt cctgtcccca aatctccctc cttctagggg
21701 ccattgtcta aagtctaaaa gaaaacatac caggcacagt ggctcatgcc
21751 tgtaatccca gcactttaaa gggagagcaa ggtaggagga tcacttgagc
21801 ctaggagttc aaaatcatca acctgggcaa cataatgaga ccctatctct
21851 acaaaaaaaa caaacaaaaa caaaaacaaa caaaaaaaac tgtaatagct
21901 gggtgtggtg gaacacactg gtagtctcag ctactcagga ggctgatagg
21951 agaaggtaac ttctgcccag gaattcaagg ctgcagtgaa ttatgattgc
22001 accactgtac tgcagcctgg gcaacagagt aagacagaga gagagagaga
22051 caaagagaaa ggaaggaagg aaagaaggaa ggagggaagg aaggaaggaa
22101 ggaaggaagg aaggaaggaa ggaaggaagg aaggaaggaa gggaagaagg
22151 aaggaaggga gggagggaag gagggaggga aagaaaggaa aggaaagaaa
22201 ataaatgtaa ctattataaa agatgttcaa tgttttattt tggaataact
22251 ttagacttac ttagaagttg caaaaatagt acagaatttc catgttcaaa
22301 tgtactcttt acctaacttt ccccaaaata ttttacctac gtatactgca
22351 attatcaaaa ccaggatatg aatacatact attagctata gtctttattt
22401 gaatttcacc agtttttaca tgcgctcaat ttttatatac actcctataa
22451 aattttctca catgtatata tgcatttaac caccaccaca atcaaaatac
22501 aaaacttttc catcacccta aataaacttg gaatgatata ctttgtatta

FIG. 1K

22551 ttcctttaca ggtactccct ctcttcaatc ctaacctctg ggaaccacta
22601 tatctgttct gtttcactgt aattttgtca tttttataat gttatataaa
22651 tgaaattgta aagtagtaat cttttgaaat tggctttttc ttacacagca
22701 tgatgctctt gagatccatc caagtgcatc aatagtgcat tcctgtttat
22751 tgcttaatag aattccatga tctagatgta acacaatttg tatattcatt
22801 tacctaatca aggtcatttg ggctgcttct agtttttggc tattacaaat
22851 aaggcagcta tgtacattca tgtgtaggtt tgtgtatgaa cgtaagtttt
22901 tatttctcta gggtcatata gtgctagttc acctttatca gaaactgcca
22951 aactatttcc ttttaaaata tagaaatgcc aatgcaaaca tgtgcatgga
23001 ggtcagcagc cccacccccc gccccacgct gccactgctg ccagtgtgaa
23051 catatgcatg gaggctggca gccccacgct caccactagc ctgcccctgc
23101 actgacactg ctaccagcat gagcgtgcac atggaggccg gcagccccat
23151 ggccaccagc actctgcccc agttgatgag cattcacccc accacactgc
23201 cacttgctct tggcactgct cttggcactt acaaatgagc acagatcctg
23251 ctgccaccac cccaacaaag tggtttggct ggcaccaccc atcagagtat
23301 tgtggccaga ggtgcaggaa caccttagcg cctccacttt agcagtttcc
23351 taacctcaag gggccagaga ataaagtcag agatccagta acagcctccc
23401 aaagttgcag catacagccc aaaagttctg agctgagcct tacccccctaa
23451 attcttccag aaatgaagcc agtcaactga acccacctta tatgagattc
23501 aaaccttcca gggcatcaaa gaagatagag gcaaaaaaaa aaaaaaaaaa
23551 aaaaaaacta tccaaaggac agcaatttca aagactgaag gaaaatcagc
23601 ccacacagat gataaagaac cagtgcaaga gctctggcaa ctcaaaagtc
23651 agagtgcttt cttacctcca aacaactgca gtagttcccc agcaatggtt
23701 cttaaccagg ctgaaatgac aaaaagagaa atcagaatgt ggataggaac
23751 agagatcact gagattcagg agacagtgaa aacctaatcc agggattcta
23801 aggattacaa caaagtgata caggagatga aagacgaaat ggccacttta
23851 agaaagaaca gaactgatct gatagagctg aaaaactcac ttcaagaatt
23901 ccagaataca atcacaaata taacggcaga atcgatcaag ctgaggaaag
23951 aatctcagag cttgaatatc gcttctctga aataactcag tcagacaaaa
24001 ataaagaaaa aaagattaaa gaagaatgaa caaaatctcc aaagtatggg
24051 attatgtaaa gacaccaaat ctatgactca ctgatgtccc tgaaagagag
24101 ggagagaaag caagcaactt ggagaacata tttcaggata tcatccatga
24151 aaattccccc aacttcacta gagaggccaa cattcaaatt gagaaaatgc
24201 agagaaccct tgtgagatac tatacagata accatcccca agacacatac
24251 ttgtcagatt ctctaaggtc aaaacaaaac aaaaaatgtt aacagcagct
24301 aaagagaagg ggcaggccac ctcccaagga aaccccatca ggctaacagc
24351 acacctttca gcagaaactc tataagccag cagagattga ggacctatat
24401 tcagcattat taaagaaaag aaattccaaa caagaatttc atatccagcc
24451 aaactaagct tcataaatga aggagaaata agattctttt caggcgagca
24501 aatgctaagg gaattcatta ccatcagacc tgccttataa gaggtcctaa
24551 agagagtgtt aaatatggaa aggaaagatt attactggca actacaaaaa

FIG.1L

24601 cgtgcttaag tacacacacc attgatgcca aaaagcaacc acacaaacag
24651 atctgcataa taaccaatta acaacacaaa acaggatttg atccttcaaa
24701 tccacacata tcaatattaa ccttaaatgt aaatgggcta aatgcccaat
24751 taaaaggcac agagtggcaa gttggacaaa gaagcaagac ccaacagtat
24801 gctgtcttga atagacccat ttcacatgca gtgacgcaca taggatcaaa
24851 ctaaagggat ggagaaaaat ctaccaagca aatggaaaac agaaaaaagc
24901 aggagttgct attctacatt caaacaaaac agactttaaa ccaacaaaaa
24951 tcaaaaaaga taaagaaagg cattacataa tggtaaaggg ttcacctcaa
25001 aaggccagac ttaactatcc taaatagata tgcatccaaa acaggagcac
25051 ccaaattcat agagcaagtt tttagacacc cacaaagaga tttagataac
25101 cacacaataa tagtgggaga ctttaacatc ccactggcag tattagacag
25151 gtcattgagg cagaaaacta acaaagaaat tcaggacctg aatttgacac
25201 ttgactaaat agacctaata gacatctaca gaaatctcca cccaaaaaca
25251 ggagtgtata tattcttctc atctgcacat ggcccataat ctaaaattga
25301 ctaatcagcc ataaaacaat ccttagcaaa taaaaaaaaa tcataccaac
25351 cacactctca gactacagtg caataaaaat agaaattaat ggaaattaac
25401 ctgcttctga atgacttttg ggtaaacaat gaaattaagg tagaaattca
25451 gaaattattt gaaactaatt agaacaaaga tactacatac cagaatctct
25501 gggacacagc taaagcaata ttaagaggga agtttataga gctgaatgcc
25551 tacatcaaaa agttagaaag atcttaagtt aacaaccgaa catcacacct
25601 agaggaaata gagaaacaag agcagatcga ccccaaagct agtagaacac
25651 aagaaatcaa aatctgcact aaactgaagg aaattgagac gcaaaaaaac
25701 atacagaaga tcaatgaatc caggacttgg ttcttcgaaa gaataaataa
25751 gatagataaa atgctagcta gactaataag gaaaaaaaga gagatcaaaa
25801 taaacacaat cagaaatgac aaagggatgt taccacctac cccacaaaaa
25851 tttaaaaaac cctcagagac tactaaaaac acctctatgc acacaaacta
25901 gaaaacctag aagaaatgga taaattcctt gaaacataca accttccaag
25951 attgaaccag gaagaaattg aatcctggac agagaccaat aatgagttcc
26001 aaaattgaat cagtaataaa aagcctacca accagaaaaa gcccaggacc
26051 agagggattc acagctgaat attacaagaa gtataaagaa gagctggtac
26101 cattcctact gaaactattc caaaaaacag aggaggaggg actcctccct
26151 aactatgaaa ccagcatcat cctgatacca aaacctggca gggacacaac
26201 aacaacaaaa aacaaaactt caggccaaat ccttgatgaa catagatgca
26251 aaaattctca ataaaatcct agtgaaatga atccagcagc acatgaaaaa
26301 gctaatccac cactatcaag gaggcttcgt ccctgggaca caagtttggt
26351 tcaacatata caaatgaata aatgtgattc atcacataaa cagaattaaa
26401 aacaaaaacc acatgatcat ctcaataaat gcagaaaagg cttttgataa
26451 agttcaacat cccttcacgt taaaaaccct caacaaacta ggcactgaag
26501 gaacatactt caaaataata agagccatct ataagaaact cacagccaac
26551 atcacagtga atgagcaaaa gctggaagca ttcttattaa acaccagaac
26601 aaaacaagga tgtcctctct caccagtcct tttcaacata gtactggaag

FIG.1M

26651 tcctggccag agcaatcagg caagagaaag atataaaagc catctgaata
26701 gaggggaagt caaactgtcc ctgattccag tcagtatgat tctatgccca
26751 gaaaacccca taatctctgc tccaaagctc cttggtttga taaacaactt
26801 cagcaaagct tcaggataca aagtcaatgt acaaaaatca gtagaatccc
26851 tgtgcaccaa caacatccaa gctgagagcc aaatcaagaa tgctatcccc
26901 caagcctgcc accatggcca cctacaaact ggtgcagatc cagcacagcg
26951 agagtgagtg gaacctggag aaccacttca gcggctggta cgacgccatc
27001 ctgagcccag cgggccatga ggcggcaaag caggcactgc gagatgctgg
27051 ctatgagttt gacatctgct tcacctcatt gcagaagaga gcgatcccga
27101 ccttctggac agtgctagat gccattgatc agatgtagct gccaatagtg
27151 aggacttggc gcctcaatga gcagcactat ggggctctag ccggtctcaa
27201 taaagcagaa actgctgcaa aacatagtga agcccaggtg aagatctgca
27251 ggtgctccta tgatgtccca ccacctccga tggagcccta ccatcctttc
27301 tacagcaaca tcagtaagga tcacaggtat gcagacctca cagaaaatca
27351 cctaccctcc tgtgagagtc tgaaggacac tattgccaga gctctgccct
27401 tctggaatga agaagtattt ccccagatca aggaggggaa atgggtactg
27451 actgcagccc atggcaacag cctctggggc actgacaagc atctgaaggg
27501 tctctctgaa gaggctatca tggagctgaa cctgccaact ggcattctca
27551 ttgtctatga attggacaag aacttgaagc ccatcaagcc cagcccacgc
27601 agttcctagg ggatgaagag accatgtgca aagccatgga agttgtggct
27651 gcccagggca aagccaagaa gtgaaggcca gcaaacaggc accctccctg
27701 cccattgcat ccatctgtcc ctccctcctg aacatgtcac actgaccaca
27751 tctatagaca tcttgagttg cagctgcaga tggggaccgg tggctcccat
27801 tttcatttta gccatttttgt cttctgcacc cactcccttc atacattcta
27851 gtcagaatag cacttctagg gcacaggttc tcagtctaag ctgtggaaaa
27901 gcccccgtta tccaagagag ttcaaagata gtgacttggg tttttgcaag
27951 tgctttgttt actaaggact tgtgaggagg agccatgctg agctacgacc
28001 aatgaggaga agcaagagag cctgtctgcc cccaggagct agtcctgtgc
28051 ttgtctgtag tcaggccact gcctgggggc tctagtcatc ccagtggaag
28101 atgaatgtaa cctgcatggt gatgtgacag ctgtttcctc cctgacccca
28201 ttacatttac ttttatttaa aaaaaaaaaa gtatagtgta tataaataat
28251 acaaaacaat aacccttcta agggtttctc gtggtggttg aaatagtccc
28301 acatgtggtc atcagaacat aagccattcc tcataccaat atggggtaag
28351 ctccttgacc tttgaggggc aggagtgctt catgctgtgt gttttagaat
28401 ccctccctgc cttgtttcat ggcagtgaaa tgcctcttgg tcctctccaa
28451 gtgtgttttt cactgatttc tgaatcatgt tgcagttgct tggccctgcc
28501 acataggtct agtgttcatt tgagcataac tgtactaaat ccttttttcca
28551 gatcagtata ataaaggagt gatgtgcaat aaaaaaaaga atgccatccc
28601 atgcacacca gttagaatgg tgatcattaa aaagtcagga aacaacaggt
28651 gctggagagg atgtggagaa ataggaacat ttttacactc ttggtgggac
28701 tgtaaactag ttcaaccatt gtggaagaca gtgtggtgat tccttaagga

Phospho-glycerate mutase, processed pseudo-gene

FIG. 1N

28751 tctagaacta gaaataccat ttgacccagc catcccatta ctgggtatat
28801 actcaaagga ttataaatca tgctgctata aagacacatg cacatgtatg
28851 tttattgcag cactattcac aatagcaaag acttagaacc aacccaaaag
28901 tccatcaatg atagactgga ttaagaaaat gtggcacgta tacaccatgg
28951 aatactatgc agccataaaa aaggatgagt tcatgtcctt tgcagggaca
29001 tggatgaagc tggaaaccat cattctcagc aaactatcac aaggacagaa
29051 aaccaaacac cacatgttct cactcatagg tgggaattga acaatgagaa
29101 cacttggatg cagggtgggg aacatcacac actggggcct gtcatggggt
29151 ggggggaagg ggggagggat agcattagga gatataccga atgtaaatga
29201 caagttaatg ggtgcagcac accaacatgg cacatgtata catgtgtaac
29251 aaatctgcac attgtgcaca tgtaccttag aacttaaagt atagtaaaaa
29301 aataaataaa taaataaaaa tttaaaaaat gaataaaata catagaaata
29351 cagctaacca ggtaggtgaa agatctctac aacaagcatt ataaaacact
29401 gctcaaagta atctgagatg atataaacaa aaggaaaagc attccatgct
29451 catggatagg aagaagcaat attgttaaaa tggccatatt gcccaaagca
29501 atttacagat tcagtgctat tcctatcaaa ctaccagttc ttcacagaat
29551 tagaaaaaaa aattatttaa aaatttgtat gaaaccaaaa aaaagcctga
29601 atagccaagg caattctaag caaaaacaac aaagccagaa gtatcacatt
29651 gtgtgacttc aatctatatt acaaggctac agtaaccaca acagcatggt
29701 actggtacaa aacacataga ccaatggaac agaagagata gcccagaaat
29751 aatgccacac acaaaccatt tgatcttaaa caaacctaac aaaaacaagc
29801 aatgggaaaa gaaattccta ttccataaat ggtgctggga taactggcta
29851 gccatatgca gaagattgaa attggacccc ttgtttatac catatacaaa
29901 aatcaactca agatgggtta aaggcttaaa cataaaacct aaaactggcc
29951 gggcacggtg gctcacgcct gtaattccag cactttggga ggccgaggca
30001 ggcggatcac gaggtcagga gatcgagacc atcctggcta acacggtgaa
30051 acgccatctg tactaaaatt acaaaaaatt agctgggcga ggtggcgggc
30101 acctgtagtc ccagctactc gggaggctga ggcaggagaa tggcgtgaac
30151 cccggggggc ggaggctgca gtgagccgag atgctgccac tgcactccag
30201 cctgggcgac agcgagactc cgtctcaaaa aaaaacaaac ctaaaactat
30251 aaaaaccctg gaagatgacc tgggaaatac cactctggac atataggacc
30301 tggcaaagat tttatgacaa agacaaaagc gattgcagca aaaacaaaaa
30351 ttgactaatt ttgtcaatta aactaaaggg atctaattaa actaaacagc
30401 ttctgcacag caaaagaaac tatcaacaga gtgaacagac aacctacaga
30451 ataggagaaa atatttgcaa actatgcatc tgacaaaggt ctattatccg
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30551 cattaaaaaa tgggcaaagt ccttgaacag acacttttaa aagaagacat
30601 acacgtggcc aagaagcata taaaaaaatg ctcaacatta ctaatcttta
30651 gagaaatgca aatcaaaacc acaataagat accatctcac aaaagtcaga
30701 atggctatta ctaaaaagtc aaaaaataac agatgctggc aaggttatgg
30751 agaaaaagga atgcttatat actgctggtg ggaatgtaaa ttagttcagc

FIG. 10